# STRUCTURE OF DEOXYRIBONUCLEOPROTEIN AS REVEALED BY ITS BINDING TO POLYLYSINE

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Received August 14, 1970

## SUMMARY

The binding of deoxyribonucleoprotein (DNP) to polylysine (PL) was studied in the hope that it might relate to the accessibility of the DNA component to RNA polymerase. PL and DNP or DNA were mixed at concentrations such that all the DNA was precipitated. Estimations were made of excess PL comprising wholly unused molecules and also unused ("wasted") lysyls on PL molecules partly bound to DNA. 100% of the DNA-phosphates and 38% of the DNP-phosphates were found to be available for binding. The "wastage" was much less for DNA than for DNP and approached zero as co-precipitation became complete. It was concluded that in DNP, much DNA is "free" though covered by protein, and lies in stretches sufficiently long to accommodate the whole of each added PL molecule.

## INTRODUCTION

The role of the chromatin proteins in control of transcription is not yet elucidated. Before determining whether removal of histones from DNA is a prerequisite for access of RNA polymerase and transcription, it seems necessary to find how much of the DNA is actually covered by protein. One approach is to study the availability of the bulk of the DNA in DNP for binding to molecules of a range of sizes. Previous work on the binding of cationic dyes by DNP has shown that about 50 - 60% of the total DNP-phosphates are unavailable (1-3). These values are surprisingly low considering the approximate numerical equality of histone basic groups and DNA-phosphate groups in the complex which could lead to almost complete mutual charge neutralization. A possible explanation is that on any one DNA molecule, there are long "free"

stretches between successive histones. However, studies on the degradation of DNP by nucleases suggested that less than 10% of the DNA exists as extensive free zones (3). Alternatively, due to the very irregular distribution of basic residues in histones (4), neutralization may be quite incomplete. Model-building studies (5) using the F2a1 fraction of known sequence, suggest that less than 60% of the DNA-phosphates lying beneath these molecules in DNP can be neutralized; the remaining 40%, though "hidden", may be accessible at least to small dye molecules.

To study the problem further, DNP-phosphates were "titrated" against PL. It was thought that a large molecule might be unable to penetrate beneath the histones to the "hidden" free phosphates. The PL titration value might therefore relate, more than the dye-binding value, to the percentage of the DNA accessible to RNA polymerase.

## MATERIALS AND METHODS

DNP was prepared from rat thymus glands as described previously (6). The final gel product, containing about 37% nucleic acid, 49% histone and 14% non-histone protein, was diluted usually to a concentration of about 0.05% DNA, using 7 x $10^{-4}$M sodium phosphate buffer, pH 6.9. Calf thymus DNA was obtained from Sigma Chemical Co. and was dissolved in the phosphate buffer to about 0.05%. Poly-L-lysine hydrobromide of average molecular weight 7,790 was obtained from Miles Seravac Co. PL was dissolved in the phosphate buffer to final concentrations of 400-1200 μM in terms of lysyl residues. Methyl orange (MeO) was recrystallized from distilled water and was dissolved in the phosphate buffer to a concentration of 1,000 μM. DNP and DNA concentrations were estimated from the value of $OD_{260m\mu}$, taking $E^{1\%}_{1cm}$ as 210. Protein concentrations were measured by a micro-biuret method (7).

## EXPERIMENTAL AND RESULTS

The procedure was to add PL to DNP or DNA at a range of concentrations. In mixtures in which more than 95% of the DNP or DNA had precipitated, excess PL was estimated by addition of excess of the anionic dye MeO (3). This dye reacts stoichiometrically with PL and precititates it: thus measurement of the optical density of the unprecipitated MeO gives the amount of excess MeO. A standard calibration curve related the optical density to the amount of PL present, i.e. in the present case to the excess PL. In the initial DNP-PL or DNA-PL mixture, excess PL could be present either as wholly unbound PL molecules or as unused ("wasted") lysyl residues in PL molecules part of which were bound to DNP or DNA. The former value was estimated by adding MeO to the DNP-PL or DNA-PL supernatant and the total excess PL by adding MeO to an equivalent but uncentrifuged DNP-PL or DNA-PL mixture.

General Mixtures of DNP or DNA and PL formed white, fibrous precipitates. With DNP-PL mixtures at molar ratios of lysyl to DNA-phosphate between about 0.38 : 1 and 1 : 1, for PL at a final concentration of about 500 μM, more than 95% of the DNP was precipitated. With DNA, the range of ratios of lysyl to DNA-phosphate producing complete precipitation of the DNA was about 1 : 1 to 2 : 1, for PL at a final concentration of about 1000 μM.

Final procedure 1 ml samples of PL at about 2000 μM were added to paired 3 ml samples of DNP at a range of concentrations (about 0.02 - 0.4% DNA). The mixtures were shaken for 30 min. One of each pair was centrifuged for 15 min at 1500 g. The supernatant was removed and its $OD_{260m\mu}$ read to check that precipitation of DNA was complete. An aliquot (usually 2 ml) was then mixed with 1000 μM MeO (usually 2 ml). Also the uncentrifuged, paired sample was mixed with MeO (usually 4 ml of each). The mixtures were shaken for 30 min and centrifuged 15 min at 400 g. The $OD_{465m\mu}$ of the supernatants, due to excess

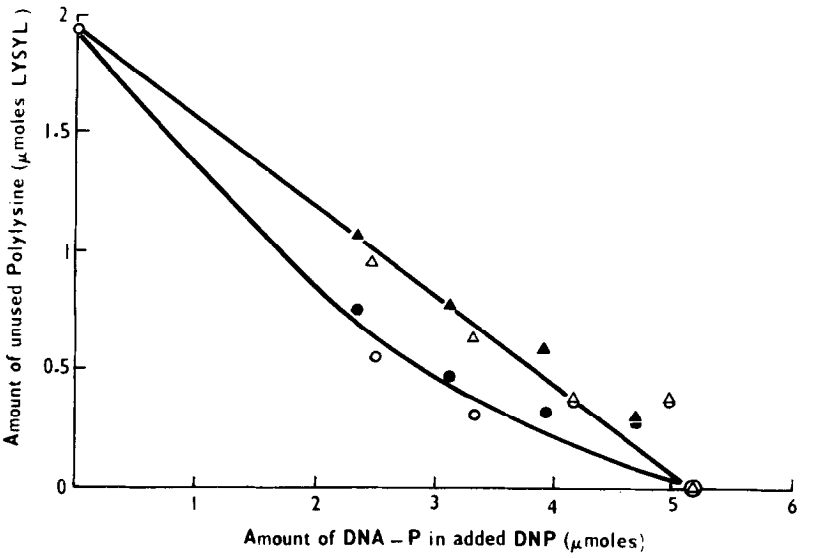


Fig. 1. Variation in amount of bound polylysine with increasing amount of DNP (values given are for 4 ml reaction mixture. Δ unused PL in DNP-PL suspension; o unused PL in DNP-PL supernatant; Δ o expt. 1, ▲ ● expt. 2

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MeO, were then read and from this the original excess PL calculated. The same procedure was used for DNA except that the initial PL concentration was usually 4000 μM and one of each pair of DNA-PL mixtures was centrifuged for 15 min at 12,000 g.

All manipulations were done at 0-4°C.

DNP plus PL Fig. 1 shows the decrease in amount of unused PL with increasing DNP concentration. Two separate experiments are shown. It can be seen that the values of unused lysyl residues in the DNP-PL suspensions (i.e. the total unused PL) decrease linearly with increasing DNA-phosphate concentration. The amount of DNA-phosphate corresponding to zero unused PL was 5.2 μmoles, giving a "titration" ratio of 38%, i.e. 38% of the DNA-phosphates are available for binding PL. At high ratios of PL to DNP, values of supernatant PL (i.e. completely unbound molecules) are much lower than the suspension values, suggesting that only part of each molecule is bound to DNP. Near the neutraliza-

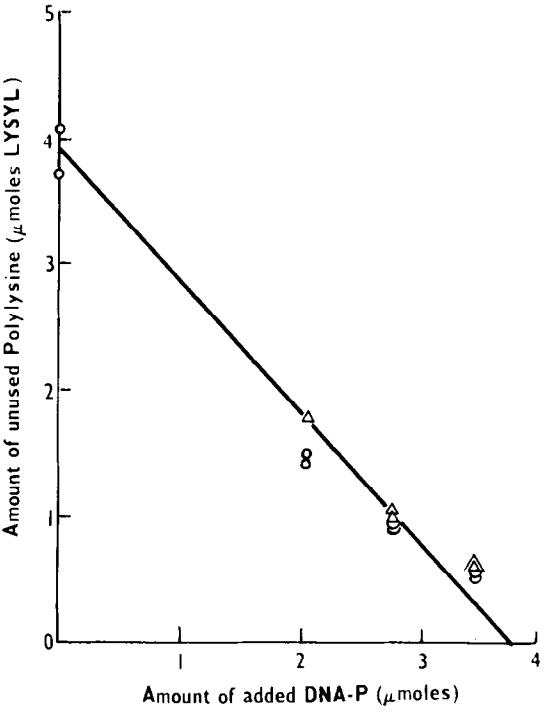


Fig. 2. Variation in amount of bound polylysine with increasing amount of DNA (values given are for 4 ml reaction mixture). Δ unused PL in DNA-PL suspension; O unused PL in DNA-PL supernatant.

tion point (at zero unused PL) the difference between the supernatant and suspension values decreases to zero.

DNA plus PL Fig. 2 shows the decrease in amount of unused PL with increasing DNA concentration. Extrapolation to zero unused PL gives a value of 104%. Table I summarizes the result for three different experiments, the mean titration value from the suspensions being 101%. The small difference between supernatant and suspension values shows that there is little wastage of lysyl residues compared with that found on mixing PL with DNP.

## DISCUSSION

The results of the "titration" of phosphates in DNA itself by PL shows that free phosphate groups can be assayed with reasonable accuracy by the poly-

Table I Estimation of DNA-phosphates available for binding polylysine (PL) of M. Wt 7,790

| Experiment | Added DNA-P (μ moles) | Unused PL (μ moles lysyl) | | PL bound (μ moles lysyl) | | Ratio PL bound/DNA-P | |
|---|---|---|---|---|---|---|---|
| | | Supernatant | Suspension | Supernatant | Suspension | Supernatant | Suspension |
| 1 | 0 | | 978 | | | | |
| | 517 | 363 | 446 | 615 | 532 | 119 | 103 |
| | 690 | 226 | 248 | 752 | 730 | 109 | 106 |
| | 860 | 132 | 146 | 846 | 832 | 98 | 97 |
| 2 | 0 | | 1140 | | | | |
| | 540 | | 542 | | 598 | | 111 |
| | 720 | | 338 | | 702 | | 98 |
| | 900 | | 143 | | 997 | | 111 |
| 3 | 0 | 778 | 760 | | | | |
| | 506 | 238 | 289 | 540 | 471 | 107 | 93 |
| | 675 | ~33 | 145 | ~745 | 615 | ~110 | 91 |

cation, using methyl orange to assess unused PL. In the case of DNP, the available free phosphates amount to about 38% of the total. This figure is surprisingly high but is unlikely to arise from any displacement of protein by PL. Garrett has found (8) that even on mixing DNP and PL under conditions more conducive to dissociation, viz in $10^{-1}$M salt, with PL at a concentration twenty times that used here, only the $F_1$ histone is displaced from the DNP. Richards and Pardon (5) have suggested that this histone fraction attaches to very little of the DNA in DNP and so its removal could scarcely affect the "titration" value.

The difference between the 38% of the phosphates in DNP available for binding PL and the 60% available for binding dyes (3) can be explained if 20% of the phosphates lie in gaps between or beneath histones too small for access by PL of molecular weight 7790. The results support the previous finding (3) that extensive truly free zones between different histone molecules on one DNA do not exist in DNP. If they did, one would expect to find, as with DNA, a low wastage of lysyl residues since the whole of each molecule could be bound within such a long free zone. Presumably wastage at high ratios of PL to DNP arises because of competition between the excess PL molecules for the comparatively limited free sites, leading to attachment of only part of each molecule. At low ratios of PL to DNP, the absence of wastage suggests that when there is no competition the average free site can accommodate the whole of every PL molecule. If the gaps were smaller than the PL molecule, only part of each could be bound to DNP at all ratios of PL to DNP, so that in contrast to the findings here, the amount of unused PL would not decrease to zero.

An alternative explanation for the absence of wastage near the neutralization point is that each PL molecule links separate gaps on the same or on different molecules. However, the error in estimation of PL near neutraliza-

tion is such that only about 5% of the total lysyl residues could be wasted but undetected; this would amount to 2 lysyls per molecule, a number unlikely to be sufficient for bridge formation.

The results suggest that long stretches of un-neutralized phosphates exist in DNP despite the coverage by protein and despite the absence of truly free zones, i.e. that much of the DNA is accessible to large molecules. The implication is that transcription can occur without actual displacement of histones from the DNA.

ACKNOWLEDGEMENT

I thank P. A. Meredith and S. M. Hammond for skilled technical assistance.

REFERENCES

1. Klein, F., and Szirmai, J.A., Biochim. Biophys. Acta, 72, 48 (1963)
2. Miura, A., and Ohba, Y., Biochim. Biophys. Acta, 145, 436 (1967)
3. Itzhaki, R.F., in preparation (1970)
4. Delange, R.J., Fambrough, D.M., Smith, E.L., and Bonner, J., J. Biol. Chem., 244, 319 (1969)
5. Richards, B.M., and Pardon, J.F., Exp. Cell Res., (in press)
6. Itzhaki, R.F., Proc. Roy. Soc. B., 164, 75 (1966)
7. Itzhaki, R.F., and Gill, D.M., Analyt. Biochem., 4, 401 (1964)
8. Garrett, R.A. Private Communication.