The Interactions of Some Cations with Deoxyribonucleic Acid Observed by the Use of Toluidine Blue as an Indicator

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There have been several studies of the metachromasy of basic dyes induced by deoxyribonucleic acid (DNA)[1,2] and of the effects of inorganic or organic cations on the metachromasy.[3-5]

We have recently observed that toluidine blue shows a marked metachromasy with DNA in solution and that this metachromasy can often be hindered by adding another substance, one which interacts with DNA, to the solution. The degree of hindrance is found to depend upon how strongly the added substance interacts with DNA. This finding suggests a simple and easy method of the semiquantitative analysis of interactions between DNA and other substances of a certain type.

As may be seen in Fig. 1, toluidine blue (5×10^{-5} mol./l.) in an aqueous soulution at pH 6 shows two absorption peaks, at 600 and 630 mμ (curve a). When DNA (from salmon sperm) is added, both of these peaks disappear, and a broad and lower peak appears at 570 mμ (curve b). The maximum metachromatic change is observed with the DNA concentration of 2.5×10^{-5} mol./l. (in *P*), i.e., with the concentration ratio [DNA]/[T. B.]=0.5. When poly-L-lysine is added to the solution, the original features of the toluidine blue spectrum is restored (curves c—e) to an extent depending upon the amount of poly-L-lysine added.

It is very probable that the metachromasy of toluidine blue occurs through its binding to the phosphate group with a negative charge of DNA. When poly-L-lysine is added to the solution, it will be more strongly bound by the DNA molecule through the N^+-H$\cdots$$^-$O—P hydrogen bond and polycationic effect than will the toluidine blue molecule, and the latter will be released from DNA. This explains the fact that its original color is restored. It is interesting that the addition of 2.5×10^{-5} mol./l. (in a lysine residue) poly-L-lysine causes a nearly complete restoration of the original spectrum of toluidine blue, because this amount is equal to that of DNA in the solution. This fact suggests that every NH_3^+ group of poly-L-lysine added is bound by a PO_2^- group of DNA.

The above simple explanation of what we have observed needs a provisory statement. Toluidine blue seems to interact slightly with poly-L-lysine. This is shown by the fact that the further addition of poly-L-lysine causes a spectrum of toluidine blue somewhat different from the original spectrum (curve a of Fig. 1.). It is quite similar in general features to the original one, but it shows a higher intensity at both peaks, 600 and 630 mμ (curves f—i in Fig. 1). The spectral change due to the slight interaction of toluidine blue and poly-L-lysine may be more clearly seen by comparing curves a and j in Fig. 1, where curve j is obtained with a mixed solution of toluidine blue and poly-L-lysine without DNA. The fact that no

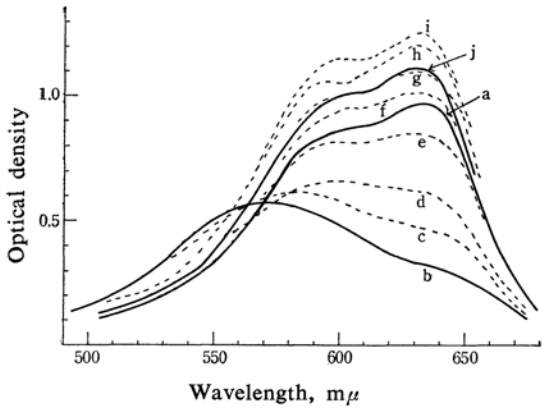

Fig. 1. Absorption curves of toluidine blue (5×10^{-5} mol./l.) in the following solvents.

a) Distilled water at pH 6
b) 2.5×10^{-5} mol./l. Na-DNA aq. soln.
c) Soln. b plus 8.0×10^{-6} mol./l. poly-L-lysine
d) Soln. b plus 1.6×10^{-5} mol./l. poly-L-lysine
e) Soln. b plus 2.4×10^{-5} mol./l. poly-L-lysine
f) Soln. b plus 3.2×10^{-5} mol./l. poly-L-lysine
g) Soln. b plus 4.0×10^{-5} mol./l. poly-L-lysine
h) Soln. b plus 8.0×10^{-5} mol./l. poly-L-lysine
i) Soln. b plus 1.6×10^{-4} mol./l. poly-L-lysine
j) 8.0×10^{-5} mol./l. poly-L-lysine aq. soln.

1) L. Michaelis and S. Granick, *J. Am. Chem. Soc.*, **67**, 1212 (1945).
2) N. Weissman, W. H. Carnes, P. S. Rubin and J. Fisher, ibid., **74**, 1423 (1952).
3) L. Cavalieri, A. Angelos and M. E. Balis, ibid., **73**, 4902 (1951).
4) A. R. Peacocke and J. N. H. Skerrett, *Trans. Faraday Soc.*, **52**, 261 (1956).
5) P. D. Lawley, *Biochim. Biophys. Acta*, **22**, 451 (1956).

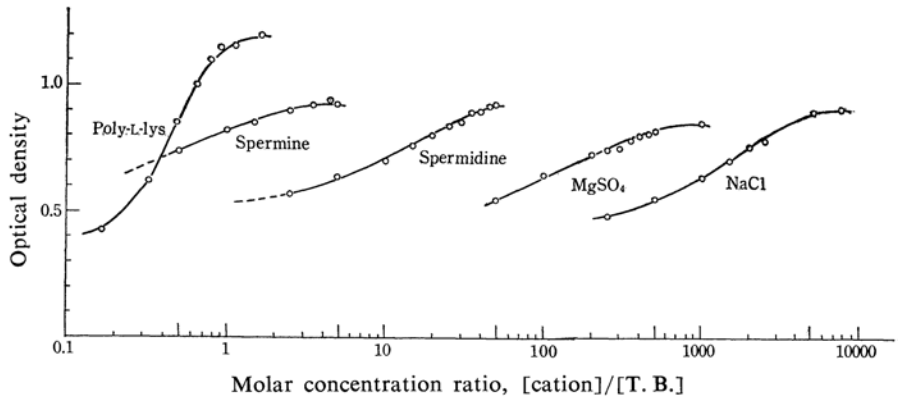

Fig. 2. Optical density of toluidine blue (at 630 mμ or at 600 mμ) in an aqueous solution containing toluidine blue 5×10^{-5} mol./l., Na-DNA 2.5×10^{-5} mol./l. and one of the substances indicated, plotted against the molar concentration ratio [added substance]/[toluidine blue]. The wavelength at which the optical density was observed is 630 mμ for poly-L-lysine, spermine, and spermidine, and is 600 mμ for $MgSO_4$ and NaCl.

distinct isosbestic point is observed in the series of the spectra given in Fig. 1 may be related to this weak interaction between toluidine blue and poly-L-lysine.

We have made similar experiments by adding spermine, spermidine, magnesium sulfate, and sodium chloride to the toluidine blue-plus-DNA system. In every case, a hindrance of the metachromasy of toluidine blue takes place at a certain concentration of the added substance. By adding a sufficient amount, a spectrum is obtained which is very similar to the spectrum of the solution of toluidine blue only. In the case of magnesium sulfate or sodium chloride, however, a slight but appreciable diminution of optical density at 630 mμ compared with the original spectrum is observed; therefore, in these cases, it would be preferable to observe and evaluate the optical densities at 600 mμ instead of at 630 mμ. Thus, the optical density at 630 mμ (or 600 mμ) gives a measure of how much toluidine blue is released from DNA and, therefore, how much the added substance is bound by DNA. This is plotted against the molar concentration of the added substance in Fig. 2. It may readily be seen that spermine is bound by DNA much more strongly than spermidine, and that Mg^{2+} is bound by DNA much more strongly than Na^+.

The Na-DNA used in the present experiment was obtained from the California Corporation for Biochemical Research, Los Angeles, and the toluidine blue, from the Merck Co., Inc. The poly-L-lysine hydrobromide, with a molecular weight of 60000, was obtained from Pilot Chemicals, Inc. The spermine tetrahydrochloride was from the Sigma Chemical Company, while the spermidine trihydrochloride was obtained from the Nutritional Biochemicals Corporation.

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