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SOME PHYSICAL PROPERTIES OF DEOXYRIBONUCLEIC ACIDS DISSOLVED IN A HIGH-SALT MEDIUM: SALT HYPERCHROMICITY

C. F. EMANUEL
Department of Physiology, University of California, Berkeley, Calif. (U.S.A.)
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SUMMARY

1. The effects of very high salt concentrations upon the u.v. absorption, viscosity, and optical rotation of DNA were studied.

2. Concentrated NaBr (7.4 *M*) caused a pronounced hyperchromicity (measured at 260 mμ) which was entirely reversible when excess salt was removed. The increased absorption (salt hyperchromicity) was approximately linear with salt concentration.

3. Heating of a DNA solution in saturated NaBr produced a further increase in absorption which was irreversible and independent of DNA concentration.

4. Salt hyperchromicity, which was most pronounced in NaBr solutions, was less evident in other halide and alkali salts, and completely absent with LiCl solutions.

5. Gradually increasing NaBr concentration produced a loss in dextrorotation of DNA from an initial value of about + 117 degrees to a final value of + 67 degrees at 7.4 *M* salt concentration. The rate of change in specific optical rotation was most pronounced in 4 to 5 *M* NaBr concentrations.

6. Concentrated NaBr solutions of DNA showed a striking loss in viscosity as compared with DNA solutions in 0.1 *M* salt.

7. Neutral salt concentration in excess of about 13 molal caused quantitative precipitation of DNA. Among the salts which were tested, precipitation of DNA resulted only from the use of LiCl solutions.

8. Some structural implications of these phenomena were discussed, and an attempt was made to compare the various types of hyperchromicity ("hydrolytic", "water", and "salt") in nucleic acids.

INTRODUCTION

It has been observed repeatedly[1-4] that a solution of deoxyribonucleic acid (DNA) in distilled water is hyperchromic in comparison with a solution of DNA in dilute salt. Although the magnitude of this hyperchromicity can exceed a value of 17 % (measured at the absorption peak of nucleic acids—260 mμ), a detailed description of the mechanics of this considerable increase in absorption is lacking. Furthermore, the phenomenon is complicated by the fact that the addition of salt to a solution of DNA in distilled water does not completely eliminate the hyperchromicity. Such a DNA sample which has been exposed to distilled water is said to be "partially denatured by dilution"[2].

A recent report from this laboratory[5] mentioned another form of hyperchromicity which was reversible. This new phenomenon occurred when the DNA was exposed to high-salt concentrations ($> 3\ M$). The present report attempts to relate these hyperchromic phenomena and to explore the effects of high-salt concentrations upon DNA in terms of u.v. absorption, viscosity, and optical rotation.

METHODS

Spectrophotometric, viscometric, and optical rotation techniques used in this study have been described[5]. All absorbancy and polarimetric measurements were made against the appropriate solvent blanks. Optical rotation measurements were carried out with the sodium illumination (589 mμ). The nucleic acid samples were isolated from spleen, kidney, and cerebellum of cattle[5,6]. These DNA samples had never been dialyzed or dried, nor exposed to ether, absolute alcohol, acids, or bases.

EXPERIMENTAL RESULTS

The hyperchromic effect of a high-salt concentration

Fig. 1 shows the relative changes in absorption spectra of DNA dissolved in various concentrations of salt. The dependency of absorption (at 260 mμ) upon the salt concentration is also evident from Fig. 2, in which it is shown that hyperchromicity begins to appear at 2 *M* salt concentrations, and reaches its maximum height in saturated (7.4 *M*) NaBr. Since the effect is also shown by NaCl and KCl, though to a lesser extent, it would seem that the phenomenon of salt hyperchromicity is due to other properties in addition to ionic strength. Further evidence that this hyperchromic effect was not caused by ionic strength alone was provided by LiCl. In this case, the absorption at 260 mμ was independent of salt concentration for any value between 1 and 6 *M* LiCl.

Whether hyperchromicity is correlated with a particular anion could not be decided with certainty since fluoride salts are too insoluble and iodide salts absorb so strongly in the u.v., that their use was precluded.

Reversibility of the hyperchromic effect

In Fig. 3, the spectra of two DNA samples dissolved in 0.1 *M* NaBr are compared. The sample portrayed in Curve A was exposed to 7 *M* NaBr for 30 min at room temperature, and then diluted to a given volume with 0.1 *M* NaBr. Curve B is the

spectrum of a sample (dissolved in 0.1 *M* NaBr) which had not been exposed to salt concentrations greater than 1 *M*. Curve C shows the spectrum of DNA dissolved in 7 *M* NaBr. Thus, the identity of Curves A and B (in comparison to Curve C) proves the reversibility of salt hyperchromicity.

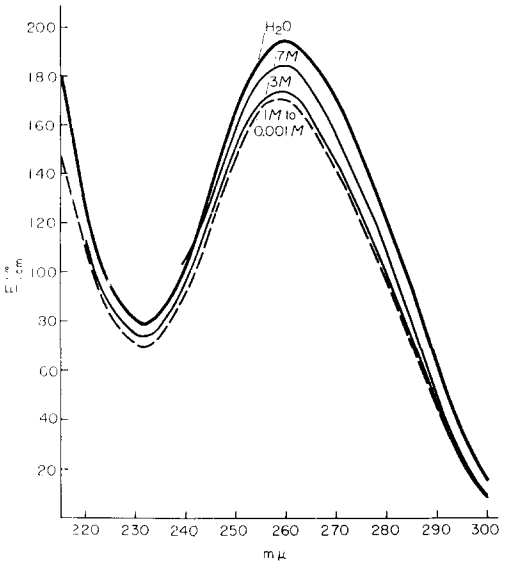

Fig. 1. U.V. absorption spectra of cattle kidney DNA as measured in various molar concentrations of NaBr.

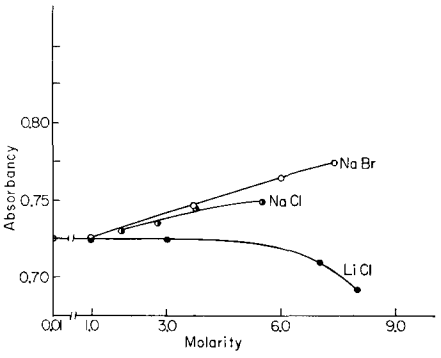

Fig. 2. Hyperchromicity of spleen DNA dissolved in various concentrations of alkali halide salts.

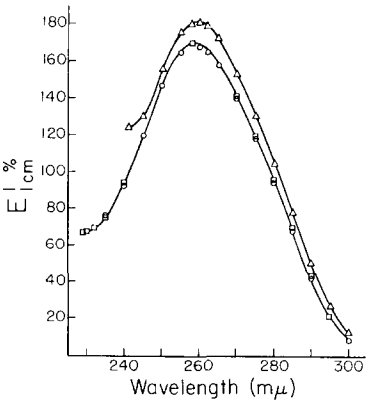

Fig. 3. U.V. absorption spectra of spleen DNA: reversibility of the salt hyperchromic effect. Spectra were measured in 7 *M*, △—△, Curve C, and in 0.1 *M*, ○—○, Curve B NaBr. A third spectrum was measured in 0.1 *M* NaBr after the concentrated DNA solution had been exposed to 7 *M* NaBr for 35 min at 25°, □—□, Curve A.

The salting-out of DNA from fourteen molal salt solution

Table I shows the approximate molarity and molality of saturated solutions of various halide salts used in this study. It was of great interest to find that DNA, like

TABLE I

MOLARITY AND MOLALITY OF HALIDE SALTS IN AQUEOUS, SATURATED SOLUTION AT 20°

Molarity, g/l; molality, g/1000 g of solvent.

Salt	*Molality*	*Molarity*
KCl	4.7	4.1
NaCl	6.2	5.5
NaBr	9.2	7.4
RbBr	6.3	5.0
RbCl	7.2	5.7
CsCl	10.6	7.2
LiCl	18.5	10.4

protein, can be quantitatively precipitated from aqueous solution by exposure to a very high salt concentration. It was determined that approx. 14 molal LiCl solution will salt-out the DNA quantitatively in a semi-fibrous form. Precipitation of DNA began at 13.2 molal LiCl concentration and was essentially complete when the molality reached a value of 13.7 (final concentration). Saturated CsCl solutions (10.6 molal) also were capable of precipitating DNA from solutions which were allowed to stand in the cold. None of the other salts used in this study were sufficiently water soluble to displace the DNA from solution.

Effect of heat on hyperchromicity

It was mentioned previously[5] that long standing of a concentrated DNA solution in 7 *M* NaBr at room temperature sometimes resulted in a flocculent precipitate or the development of a slight opaqueness of the solution. Since a similar degradation process also takes place in the cold (but more slowly), it was of interest to determine the effects of heating on such solutions. Fig. 4 demonstrates that heat (100°) produces an almost immediate degradation reaction which further heating for as long as 20 min does not change (as measured by absorbancy at 260 mμ). Heating not only caused an increase in absorbancy at 260 mμ (Fig. 5) but also caused the nucleic acid to become very sticky and viscous. Most samples remained transparent under these conditions of limited heating; eventually, an opaqueness or precipitate developed. Fig 6 shows

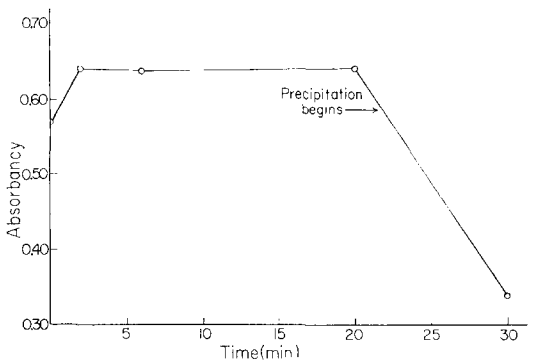

Fig. 4. Relationship between the duration of heating a solution of kidney DNA in saturated NaBr and the resultant absorbancy at 260 mμ.

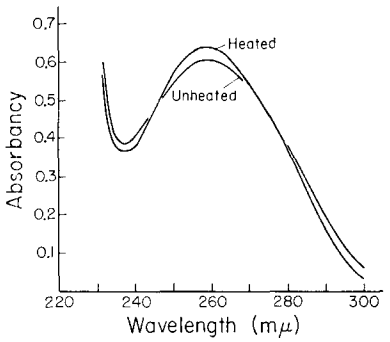

Fig 5. U.V. absorption spectra of cerebellum DNA heated in 7 *M* NaBr. Samples (100 μg) of DNA dissolved in 7 *M* NaBr were heated 5 min at 100° and diluted to 5 ml with water.

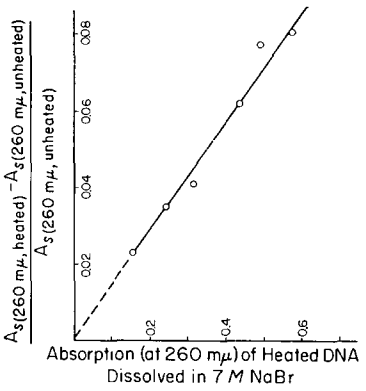

Fig. 6. Absorption increment at 260 mμ of heated kidney DNA dissolved in 7 *M* NaBr.

that the degradation process (which results when a DNA solution in 7 *M* NaBr is heated) is independent of nucleic acid concentration. The slope of this "degradation curve" was not reproducible from one DNA preparation to the next.

Viscosity changes

Many studies have shown the effects of salt on the viscosity of DNA solutions. In this regard, CONWAY AND BUTLER demonstrated that 0.1 *M* NaCl not only depressed the viscosity of a DNA solution, but also caused the viscosity numbers to be nearly independent of DNA concentration[7]. These authors also pointed out that the concentration-dependent curve for the viscosity of DNA in water, when extrapolated to zero concentration of DNA, would yield viscosity numbers similar to those obtained for DNA dissolved in 0.1 *M* salt.

In Fig. 7 I have compared the viscosities obtained in 7 *M* NaBr with those obtained in dilute KCl. It is evident that very high salt concentrations caused a marked viscosity depression which, incidentally, facilitated the filtration of DNA solutions during isolation procedures.

Optical rotation

Earlier investigations[8,5] revealed that dilute salt solutions of nucleic acids have positive rotations in excess of + 100 degrees. Almost the whole of this positive

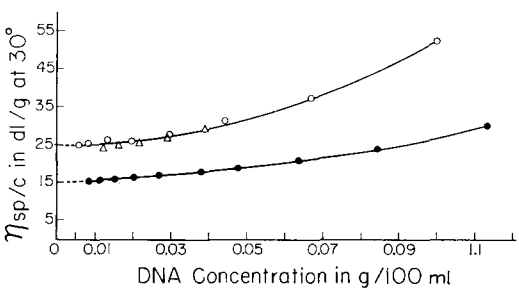

Fig. 7. Effect of high-salt concentrations upon viscosity of DNA solutions. ●, cattle cerebellum DNA dissolved in 7 *M* NaBr; ○, in 0.02 *M* potassium arsenate buffer, pH 7.0; △, in 0.1 *M* KCl.

value presumably relates to the uncompensated helical structure of the DNA molecule since an equimolar mixture of the constituent four nucleotides arithmetically averages to a negative value* (approx. $-38°$). Fig. 8 demonstrates that 7 *M* NaBr causes a decrease in the specific rotation to a value of about $+ 65°$, and that the value attained is constant for at least 20 h at 30°. Fig. 9 shows the effects of a gradually increasing salt concentration upon DNA specific rotation. It was evident that the optical rotation values began to decrease at the 0.1 *M* NaBr concentration, and reached a lower limiting value in the 4 to 6 *M* NaBr concentrations.

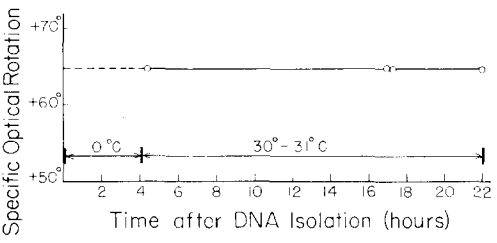

Fig. 8. Effect of time upon specific optical rotation of spleen DNA dissolved in 7 *M* NaBr.

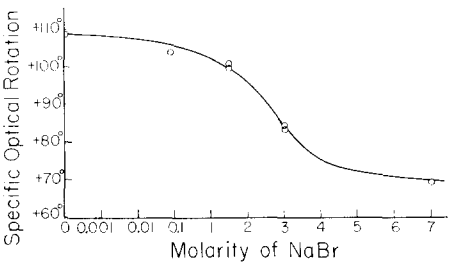

Fig. 9. Effect of salt concentration upon the optical rotation of spleen DNA.

DISCUSSION

The advantages which arise from the use of saturated sodium bromide in preference to molar sodium chloride for DNA isolation were discussed in an earlier publication[5]. Unfortunately, we know very little about the effects of a very high salt concentration upon the integrity of the DNA molecule. In the present study, the observed spectrophotometric, viscometric, and polarimetric changes caused by saturated NaBr were found to be reversible and, therefore, within the limits of these procedures, high-salt concentrations do not have irreversible degradative effects.

The protective effect of dilute salt upon DNA structure as well as the hyperchromic reaction of DNA exposed to distilled water have been widely studied. The present investigation of the effects of a high-salt concentration demonstrated that another hyperchromic shift appeared when the molarity exceeded a value of two. Thus, the hyperchromic phenomena of nucleic acids can be classified under three headings: (a) Hyperchromicity arising from degradation procedures (*e.g.*, acid, alkali, enzymes; this type (referred to here as "hydrolytic" hyperchromicity) is irreversible. (b) Hyperchromicity arising when a DNA solution in dilute salt is transferred (by dialysis) to distilled water. This partially reversible effect, called "water hyperchromicity", is known to inactivate the transforming activity of microbial nucleic acids[10], and the extent of degradation which accompanies water hyperchromicity is dependent upon the DNA concentration[2,11]. (c) "Salt hyperchromicity", which appears to be completely reversible[5].

* In this regard, it was of interest to find that the treatment of a DNA solution with pancreatic nuclease reduced the optical rotation (specific) from an initial value of $+ 110°$ to a final value of $+ 12°$ (measured in 0.08 *M* potassium arsenate buffer). When this DNA digest, known to contain oligo-nucleotides[9], was measured in 7 *M* NaBr, the specific rotation was depressed further to $+ 7.9°$. Treatment of the initial DNA digest with intestinal phosphatase reduced the specific rotation (in 0.08 *M* arsenate buffer) to $+ 5°$.

These three expressions of hyperchromicity provide evidence that the complex structure of the DNA molecule can become altered in a manner which permits each purine and pyrimidine chromophore to contribute a greater share of its potential absorptive capacity to the chromophore system of the entire molecule. The maximum hyperchromic effect is produced when the nucleic acid is completely degraded by hydrolysis to the four constituent nucleotides (or purines and pyrimidines); this maximum increase in absorption can be as great as 40 %. Water hyperchromicity produces a 17 % increase in absorption and involves the breakage of hydrogen bonds only (some of which can re-form under suitable conditions). Salt hyperchromicity, which constitutes a 12 % increase in absorption, probably results, from limited cleavage of hydrogen bonds, all of which can re-form when excess salt is removed.

Application of the general rule[12], that lengthening of the chromophore enhances the total absorbancy of a system, leads to the prediction that, in the "native" DNA molecule, the chromophore interaction must be of a type that reduces the individual contribution of each heterocyclic chromophore. Present chemical knowledge of DNA structure indicates that there can be no interaction of the chromophores through covalent conjugation (*i.e.*, through the sugar-phosphate diester bonds). Thus, we are led to believe that the close proximity of the heterocyclic bases, stacked one above the other, results in an extension of the electron orbitals over more than one nucleotide[13]. Normally, this interaction must be such that each chromophore is unable to contribute its full capacity in the absorption of u.v. light—perhaps due to the fact that each nucleotide is slightly displaced along the helix (see ref. 14, p. 462), and does not lie directly over or under its neighbouring nucleotides. An alternative explanation is that these losses in absorption may be the result of shielding (see ref. 14, p. 488).

However, if the DNA helix could be forced to expand lengthwise, there would be less inhibitory action between the chromophores because of the greater internucleotide distance, and each base would be able to contribute a larger share to the total absorption. This possibility for explaining water hyperchromicity is supported by ROWAN's light scattering and flow birefringence studies wherein he showed that the DNA molecule lengthens as much as 38 % when transferred from dilute salt solution to pure water solution[15]. This lengthening is generally assumed to result from internucleotide charge repulsion. MICHELSON's data[13] demonstrate that even certain dinucleoside phosphates exhibit a marked hyperchromicity upon hydrolysis. Again, this indicates that the hyperchromic phenomena must result primarily form chromophore interaction between adjacent heterocyclic bases rather than through interaction of the chromophores in adjacent chains of the DNA double helix.

The experimental fact that the extensive lengthening of the DNA helix (as occurs in distilled water solutions) is accompanied by a hyperchromic shift leads me to suspect that salt hyperchromicity is also a consequence of an expansion of the molecule. An expansion necessitates an increase in helical pitch, and thereby, a loss in specific dextrorotation. As shown in Fig. 9, this prediction is fulfilled (specific optical rotation falls from an initial value of $+ 117^\circ$ to a final value of $+ 67^\circ$). However, this interpretation of the relation between salt hyperchromicity and the decrease in dextrorotation is open to question upon the following grounds: (a) The decrease in dextrorotation is not a linear function of salt concentration—the maximum rate of change being observed in the 3 to 4 M region—whereas salt hyperchromicity increases in a linear fashion between 1 and 7.4 M NaBr concentration. (b) A decrease in optical

rotation would also be expected when the DNA is transferred from dilute salt to distilled water. Such a decrease was not observed (Fig. 9).

It must be admitted that the manner in which a high-salt environment could cause a reversible extension of the helix is not clear. This problem is further complicated by the fact that different neutral salts produce different degrees of hyperchromicity and, in the case of LiCl, no trace of salt hyperchromicity was observed. Also, the effect does not appear to be correlated with the qualitative nature of either cation or anion. Among the salts tested, sodium bromide produced the most striking hyperchromicity.

When the DNA solution in saturated NaBr was heated to 100°, an almost immediate irreversible denaturation occurred. The DNA became very viscous, the hyperchromicity increased even more, and the DNA sometimes precipitated. These effects probably were the result of separation of the DNA double helices followed by collapse to a disoriented structure[16].

One of the important advantages arising from the use of saturated NaBr (9.2 molal) for DNA isolation results from the salting-out and removal of protein, including nucleases, from the DNA preparation[5]. However, exposure of DNA to neutral salt concentrations in excess of about 13 molal caused salting-out of the DNA itself. Among the salts tested, this effect was produced only with LiCl (at room temperature). Precipitation of DNA in this manner was probably the result of molecular aggregation which followed the removal of the water sheaths (ice structure) surrounding the DNA helices[17]. The potential usefulness of this precipitation method for separation of ribonucleic acid from DNA, for DNA isolation and fractionation, is now being investigated.

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