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DEOXYPENTOSE NUCLEIC ACIDS

XI. THE DENATURATION OF DEOXYRIBONUCLEIC ACID IN AQUEOUS SOLUTION: CONDUCTIVITY AND MOBILITY MEASUREMENTS

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SUMMARY

Various electrical properties have been derived from conductivity and electrical transport experiments for aqueous solutions of DNA. The specific conductivity shows a discontinuity when plotted against DNA concentration. Below this critical concentration the material transport number falls rapidly with decreasing concentration. The critical concentration corresponds to a similar discontinuity observed in the variation of O.D. with DNA concentration, measured at 2590 Å. A possible explanation of this phenomenon involves the denaturation of DNA at concentrations below the critical concentration. Above this concentration zone native DNA predominates with a charge fraction of 0.4 while below this concentration denatured DNA exists with a very high charge, but lowered mobility.

Abbreviation: DNA, deoxyribonucleic acid.

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INTRODUCTION

Although it has now been well established that DNA in solution behaves as a relatively stiff coil and does not undergo the morphological changes with ionisation observed with synthetic polyelectrolytes, variations of the charge of the DNA ion must produce some significant changes in flexibility. It is therefore desirable to know the charge on the nucleate ion under various conditions of ionic strength and pH.

The charge on the DNA ion has previously been determined from either membrane potential measurements or from the electrophoretic mobility. Both these methods are subject to criticism, the first because of the high DNA concentrations required to obtain measurable potentials and the lack of reproducibility of the results, the second in that the calculation of charge from electrophoretic mobility measurements requires assumptions concerning the size and shape of the kinetic unit. Values of the charge fraction (net charge per phosphorus atom) obtained from membrane potential measurements[1,2] vary from 0.4 to 0.5 at high ionic strength (0.1 or 0.2) to 0.2 at ionic strength approaching zero. The electrophoretic measurements[3] indicate a value of 0.2 which appears to be almost independent of the ionic strength. These values of the charge show that the net negative charge is less than the theoretical value of one electronic charge on every phosphorus atom, a result which has been explained by the association of sodium ions into the DNA kinetic unit. Similar behaviour has been observed with solutions of synthetic polyelectrolytes[4–7] and also for bovine serum albumen[8].

In this investigation the charge on the nucleate ion has been determined from conductivity and electrical transport measurements, using the methods described by HUIZENGA, GRIEGER AND WALL[4] for a study of the polyacrylate ion. The electrical properties of DNA solutions were studied as a function of DNA concentration in the absence of added electrolyte and, as was to have been expected from the observed denaturation of DNA at low ionic strengths[9–11], the results of this investigation are complicated by the presence of a denaturation phenomenon.

EXPERIMENTAL

The DNA used in this investigation was prepared from calf thymus glands by the detergent method[12]. The preparations were the salt free samples 2, 3, 4 and 7 which have been described in an earlier investigation[13]. The atomic extinction coefficients of these samples, measured in 0.1 M NaCl at 2590 Å, were found to be 6650, 6650, 6710 and 6610 respectively. The absence of sodium chloride in preparation 2, which was the preparation mainly used in this investigation, was confirmed by analysis. Solutions of DNA were prepared by dissolving the solid DNA in water at 4° to give a final concentration of $3 \cdot 10^{-3}\,M$ with respect to phosphorus. The various dilutions were made by the addition of water to the stock solutions. All concentrations were determined by phosphorus analysis on each stock solution.

A conductivity bridge, without a Wagner earthing system, was used. The oscillator was isolated from the bridge by an astatically wound shielded transformer and, since the supply was not resistively connected to ground, one side of both the cell and the bridge could be earthed. Measurements were made at 1 kc/sec. The water used through-

out has a specific conductivity of $1 \cdot 10^{-6}$ ohm^{-1} cm^{-1}. All measurements were made at $25.00 \pm 0.05°$.

Transport measurements and the calculation of the derived electrical properties were made using the method described by HUIZINGA, GRIEGER AND WALL[4]. Endosmotic flow was eliminated by an opposing pressure and the changes in DNA concentration in the electrode compartments were determined by phosphorus analysis. Transport measurements were made on sample 2 only. All electrical properties refer to the Grund molecular unit.

RESULTS AND DISCUSSION

The variation of the specific conductivity and material transport number (the ratio of increase of DNA phosphorus in anode compartment to the total equivalents of electricity passed) with DNA concentration are shown in Fig. 1. The specific

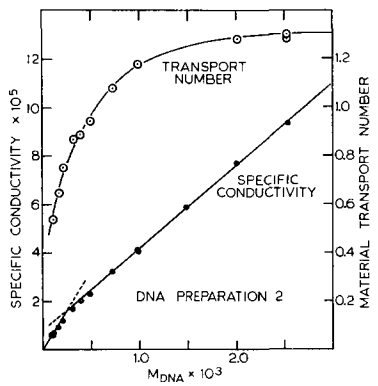

Fig. 1. The specific conductivity and material transport numbers of aqueous DNA solutions at various concentrations.

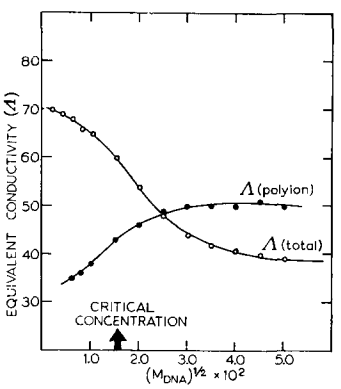

Fig. 2. The total equivalent conductivity and the nucleate ion equivalent conductivity in aqueous solution.

conductivity, as a function of DNA concentration, shows a definite discontinuity over a short range of concentration at $2\text{–}3 \cdot 10^{-4}$ *M* DNA. The material transport number rises rapidly at concentrations above the discontinuity and finally levels off at a value greater than unity. Material transport numbers greater than one infer that a proportion of the sodium ions are associated so strongly with the polyion kinetic unit that they move with it in an electric field thus producing a negative sodium ion material transport number. This result is in agreement with the earlier deductions concerning gegenion association with DNA[1–3] and with partially neutralized polyacrylic acid[4]. It can be seen that the gegenion association process only becomes apparent at concentrations above the discontinuity (in future designated as the critical concentration).

These results were combined, using smoothed electrical transport values and average values of the specific conductivity from preparations 2,3, 4 and 7, to derive the more fundamental electrical properties. In Fig. 2 the variation of the total and polyion equivalent conductivities with concentration are compared. Although the total equivalent conductivity decreases with increase of concentration above the

critical concentration, the contribution from the polyion unit increases. This result may also be attributed to the association of sodium ions with the polyion. The contribution of the sodium ions to the conductivity of the solution is thus decreased and there is therefore a net reduction in the total equivalent conductivity. The changes that occur in the charge fraction with changing DNA concentration are shown in Fig. 3. A large increase in charge occurs on decreasing the DNA concentration below the critical value and there is a simultaneous decrease in the mobility in this concentration range. The mobility, however, in this case, is not dependent on the charge carried by the kinetic unit (mobility $\propto$ polyion equivalent conductivity/charge fraction), but is a true indication of the frictional forces opposing the movement of the polyion unit in solution.

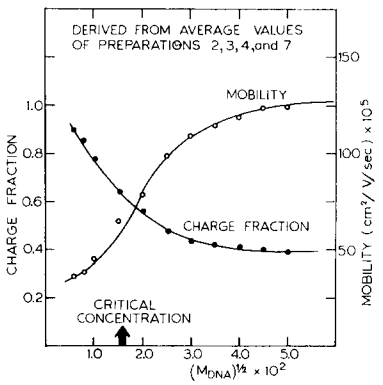

Fig. 3. The charge fraction and mobility of aqueous DNA solutions at various concentrations.

Similar changes to those described for DNA have been found for detergent solutions[14–16] which display a critical micelle concentration (CMC). The increased mobility above the CMC has, in this case, been explained in terms of aggregation such that the aggregate units (micelles), which form above the CMC, experience less frictional drag than do the same number of molecular components in the unaggregated state. Notwithstanding the improbable shape changes that native DNA would have to undergo to produce the changes in electrical properties described above, it would appear that some form of aggregation or disaggregation phenomenon is also in operation in the case of aqueous DNA solutions. Two alternative explanations appear possible.

1. The aggregation process may be similar to that found for detergent ions. Two or more DNA units would aggregate in such a way that the resulting kinetic unit offers less frictional resistance than that experienced by the constituent DNA units separately. Such a process should result in a reversible single molecule–aggregate system, because there is no reason to suspect that these molecules would be aggregated by a specific type of linkage. This process would also involve a molecular weight change at the critical concentration.

2. The aggregation phenomenon might be of entirely different origin if the intact native DNA unit is classed as the aggregate. Passage through the critical concentration on decreasing the DNA concentration could then represent the disaggregation of the native DNA double helix by hydrogen bond cleavage between the paired nitrogen

bases. This would eliminate the force holding the two spirals in register, and the molecule would then be in what is known as the denatured state. As the hydrogen bonds between the base pairs are thought to be highly specific, such a disaggregation process described above should be quite distinct from the former process (1) in being irreversible. Some ambiguity arises when the molecular weight changes are considered. Once the specific hydrogen bonds have been broken, there is no direct linkage between the two spirals, but, due to the plectonemic nature of the interwound strands, no decrease in molecular weight can immediately take place. In the case of denaturation by acid[17], alkali[18], thermal treatment[19] and low ionic strength[11], no change in molecular weight has been found (when measured at high ionic strength), as compared with the native sample. On the other hand, once all the hydrogen bonds have been broken, unwinding of the two chains is apparently energetically feasible[20]. It is possible that one of the more important factors which determine whether the spirals do unwind is the presence of a few remaining native hydrogen bonds or of some reformed hydrogen bonds of a non-specific nature. However, by analogy with acid, alkali, thermal, and low ionic strength denaturation, it would be expected that process (2) would proceed without molecular weight change.

It is thus evident that the two possible explanations given above for the critical concentration phenomenon can be differentiated by the criteria of reversibility and possibly molecular weight change.

Further, experimental evidence supports the finding that some structural change occurs in the kinetic unit on diluting below the critical concentration zone. The variation of O.D. (measured at 2590 Å) with DNA concentration shows a discontinuity[11,21,22] similar to that found for the conductivity of DNA solutions. This discontinuous optical property has been confirmed in the present investigation and the value of the critical concentration agrees with that found from the conductivity measurements. The discontinuity in the u.v. absorption is such that below the critical concentration the extinction coefficient increases.

In view of the fact that denaturation has already been shown to occur on reducing the ionic strength[9,10] and by dissolution in water at low DNA concentrations[11], it would appear that the mechanism (2), involving hydrogen bond cleavage, is the more likely explanation of the critical concentration phenomenon. The increase in extinction coefficient on dilution below the critical concentration would also be compatible with a denaturation process.

If the critical concentration phenomenon does arise from a denaturation process, then the electrical properties described above will refer to native DNA at high concentrations only. Under such conditions the value of the charge fraction is 0.40 (Fig. 3) for the native material in the absence of added electrolyte. This is to be compared with the values previously reported of 0.2 and 0.4 at zero or low ionic strength[1-3]. The denatured material (at concentrations below the critical zone) apparently has a much higher charge, which approaches the theoretical maximum value. The mobility of the denatured material has decreased as compared with the native DNA. Although the charge increases on passing from the native to the denatured state, the equivalent polyion conductivity decreases; this arises from the large mobility decrease which accompanies this process.

Although it would be expected that denaturation would be accompanied by a general molecular collapse, as indeed has been shown for denatured DNA in electrolyte

solutions[11] at high ionic strength, this does not appear to be the case for denaturation caused by dilution in salt free solutions. It has been found that denaturation in aqueous solution leads to a highly charged unit and it is thought that, in this instance, an intramolecular repulsion acts counter to the collapsing tendency brought about by the rupture of the hydrogen bonds. The large size of the kinetic unit could possibly explain the low mobility observed for denatured DNA.

Further investigations will be necessary to determine whether denaturation by dilution of aqueous DNA solutions can completely explain all the electrical properties reported above.

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