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DEOXYRIBOSENUCLEIC ACIDS

X. THE SHAPE OF THE SEDIMENTING BOUNDARY OF SODIUM DEOXYRIBONUCLEATE

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SUMMARY

The formation of a hypersharp boundary during the sedimentation of sodium deoxyribonucleate in electrolyte solutions has been investigated using a synthetic boundary cell. Hypersharp boundaries are formed between solutions possessing not greatly different sedimentation coefficients and it is thus concluded that the formation of a hypersharp boundary cannot be solely attributed to the concentration dependence of the sedimentation coefficient as generally assumed. A tentative explanation based on the variation of charge with concentration is put forward.

INTRODUCTION

One of the most striking features of the sedimentation behaviour of sodium deoxyribonucleate (DNA) is the hypersharp boundary that is always observed for a sample which is relatively undenatured, both in the presence and absence of added electrolyte. A typical hypersharp boundary for DNA is shown in Fig. 1. The phenomenon of boundary sharpening is well known in both electrophoresis and sedimentation. In sedimentation, the boundaries are found to be less spread than would be expected from a knowledge of the sedimentation coefficient distribution and the diffusion coefficient and this behaviour has usually been regarded as a consequence of the concentration dependence of the sedimentation coefficients of the solutes. BALDWIN[1]

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and FUJITA[2] have shown that the shape of a sedimentation boundary may be expressed in terms of the distribution of the sedimentation coefficients at zero concentration and of the concentration dependence of the sedimetation coenfficients of the solutes, provided that the latter is a linear function and that diffusion may be neglected. Two distinct, but closely related effects are recognised by BALDWIN, viz., the dependence of sedimentation coefficient upon concentration and the mass transport resulting from the consequential dependence of concentration on sedimentation coefficient. The latter was first recognised and formulated by JOHNSTON AND OGSTON[3].

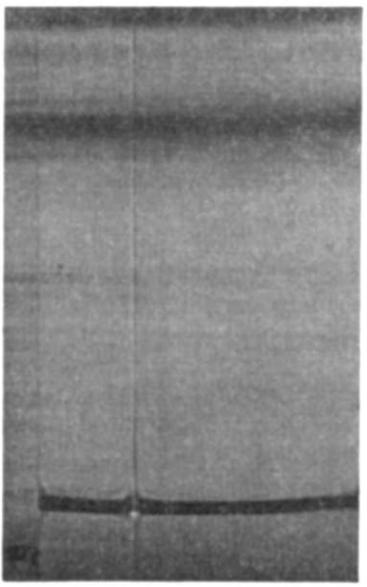

Fig. 1. Typical hypersharp boundary for DNA.

When solutions of linear polymers and proteins exhibit a negative dependence of the sedimentation coefficient on the concentration, as predicted theoretically by GOLDBERG[4] and in general observed experimentally, BALDWIN has shown that both the effects considered lead to an increase in the sharpness of the boundaries formed at finite concentrations over that of the hypothetical boundary that would exist at zero concentration. Furthermore, BALDWIN has shown that in the particular case of a sample of commercial dextran the shape of the sedimenting boundary is determined very largely by the concentration dependence of the sedimentation coefficient and that the influence of the JOHNSTON-OGSTON effect is very small. In view of this observation, it is proposed to consider, in this communication, the shape of the sedimenting DNA boundaries with reference only to the concentration dependence of the sedimentation coefficient thus neglecting the JOHNSTON-OGSTON effect.

The hypersharp boundary exhibited by DNA is much more sharp than most other sedimenting boundaries, except for those formed during the sedimentation of high molecular weight synthetic polyelectrolytes sedimenting in the absence of added electrolyte. These hypersharp boundaries have always been assumed to be a consequence of the high concentration dependence of the sedimentation coefficient. This

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assumption, however, appears to be untenable in view of the sedimentation results obtained by HOWARD AND JORDAN[5] on fully neutralised polymethacrylic acid (PMA). These authors found that a hypersharp boundary was formed if sedimentation was carried out in the absence of added electrolyte, whereas if sodium chloride was added to the solution, a spreading boundary was obtained. However, the concentration dependence of the sedimentation coefficient of PMA is considerable and not greatly affected by the presence of sodium chloride; on the basis of the concentration dependence hypothesis, therefore, hypersharp boundaries should be formed both in the absence and presence of sodium chloride which is contrary to the experimental observations. Furthermore, STEINER AND BEERS[6] have shown that at concentrations of poly-adenine greater than 0.2 % in 0.1 *M* KCl, hypersharp boundaries are obtained, but that at lower concentrations, spreading boundaries are observed even though the concentration dependence of the sedimentation coefficient is increased. This observation is contrary to the concentration dependence hypothesis although at higher ionic strengths, increase of boundary spreading occurs with a decreased concentration dependence in agreement with the hypothesis.

A direct method of testing the concentration dependence hypothesis is to form a synthetic boundary between 2 concentrations of solute such that the rates of sedimentation on either side of the boundary are not greatly dissimilar. Under these conditions, and if the concentration dependence hypothesis were correct, the synthetic boundary would not be hypersharp. This technique has been employed for solutions of DNA, PMA and polyvinyl-*n*-butyl-pyridinium bromide (PVPB).

EXPERIMENTAL

The DNA was prepared by the method of KAY, SIMMONS AND DOUNCE[7] from calf thymus glands which were frozen on removal from the animal. The dried material contained sodium and phosphorus in a 1 : 1 molar ratio and possessed an ε_p value of 6530 at 259 mμ and of 2580 at 231 mμ (where ε_p is the extinction coefficient/mole of phosphorus). According to the criteria suggested by CHARGAFF[8], this preparation of DNA may be regarded as undenatured.

Concentrations of solutions of DNA were determined by u.v. absorption measurements. Standardisation was carried out by heating a sample of DNA to constant weight under vacuum at 110° and dissolving in a weighed quantity of water. From this solution, a number of different solutions were prepared by dilution by weight and solid sodium chloride added to make the solutions 4 % by weight with respect to the sodium chloride. The O.D. of the solutions at 259 mμ were then determined. From the relationship between concentration, in terms of weight of dried DNA, and the O.D. at 259 mμ, the concentration of any other solution could be determined, after it had been made 4 % with respect to sodium chloride.

PMA was prepared by Mr. J. E. A. GOODEN, M.Sc., using a ferrous ion–hydrogen peroxide initiator in aqueous solution, freed of iron impurity, fractionated by the addition of methylethyl ketone, neutralised with sodium hydroxide and the solid sodium salt isolated by freeze drying.

PVPB was prepared by Dr. T. KURUCSEV by quaternising a sample of poly-4-vinyl pyridine prepared by JORDAN, MATHIESON AND PORTER[9].

Sedimentation measurements were carried out on a Spinco model E ultracentri-

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fuge equipped with Schlieren and u.v. optical systems. Sedimentation was performed at 59,780 rev./min (A rotor) and 50,740 rev./min (B rotor). Synthetic boundaries were formed by means of Spinco synthetic boundary cell. Solution temperatures were determined by measuring the rotor temperature with a thermocouple before and after each sedimentation. The mean of the 2 temperatures was corrected for the adiabatic cooling of the rotor. The temperatures obtained in this way were used to correct the observed sedimentation coefficients to 20°.

The Schlieren pattern photographs were measured using a Pye 2-dimensional measuring microscope reading to 0.01 mm. The hypersharp boundaries usually exhibited by DNA show no true maximum, only a vertical line which under the microscope is found to be a diffraction pattern; measurements were always made to the apparent centre of this diffraction pattern. The values obtained for the 2 lowest concentrations in Fig. 2 were obtained using u.v. optics, the absorption photographs were measured on a Spinco Analytrol with microanalyser attachment. Measurement was made of the movement of the centre of the boundary which at these concentrations was not hypersharp.

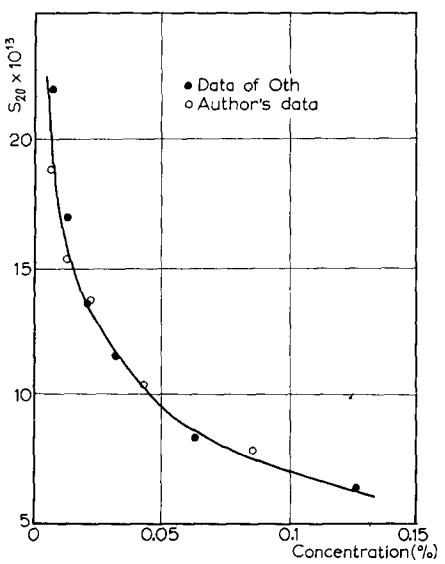

Fig. 2. Variation of sedimentation coefficient of DNA with concentration.

RESULTS AND DISCUSSION

In Fig. 2 is shown the variation of S_{20} for DNA with concentration in 0.2 *M* sodium chloride taken from the measurements of OTH[10] together with some results of the authors. The variation of S_{20} with concentration increases sharply below a DNA concentration of 0.04%, but between 0.05 and 0.10% the variation of S_{20} with concentration is not great. In order to test whether the concentration hypothesis for the formation of hypersharp boundaries is correct, a synthetic boundary was formed in a synthetic boundary cell between DNA solutions of concentration approx. 0.05 and 0.10% in 0.2 *M* NaCl. The sedimentation patterns obtained are shown in

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Fig. 3, where it can be seen that the synthetic boundary is just as hypersharp as the conventional boundary which is formed simultaneously at the top of the cell.

The rates of sedimentation in the various regions of the cell are shown in Table I for 2 typical expts. It can be seen that the difference in sedimentation rates between each side of the synthetic boundary is about 1/6 of the difference for the conventional boundary, yet each appears equally hypersharp. These results indicate that the hypersharp boundaries observed with DNA cannot be the direct result of the solute on the low concentration side of the boundary tending to overtake the slower moving material on the high concentration side, although this effect may contribute to a small unknown amount.

TABLE I

SEDIMENTATION COEFFICIENTS OF DNA IN VARIOUS REGIONS OF THE CELL DURING SEDIMENTATION

Experiment	*Sedimentation coefficient (S_{20})*		*Difference*
	Above boundary	*Below boundary*	
Conventional boundary, between 0 and 0.05 % DNA			
C_1	approx. 21	9.1	12
C_3	approx. 21	10.1	11
Synthetic boundary, between 0.05 and 0.1 % DNA			
C_1	9.1	6.6*	2.5
C_3	10.1	8.3*	1.8

* The rates of sedimentation below the synthetic boundary were calculated using the equation given by BALDWIN[11].

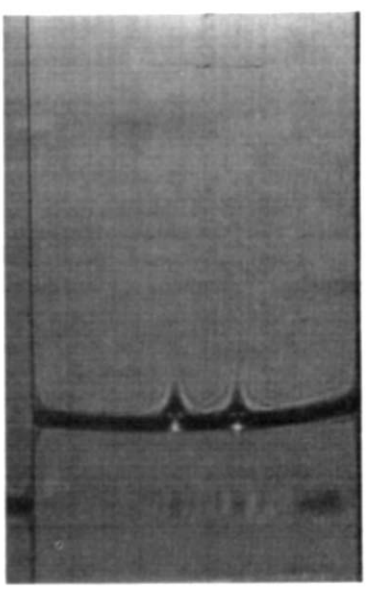

Fig. 3. Sedimentation of DNA in 0.2 *M* NaCl using synthetic boundary cell. Left hand boundary between zero and 0.05 %, right hand boundary between 0.05 and 0.10 % solutions.

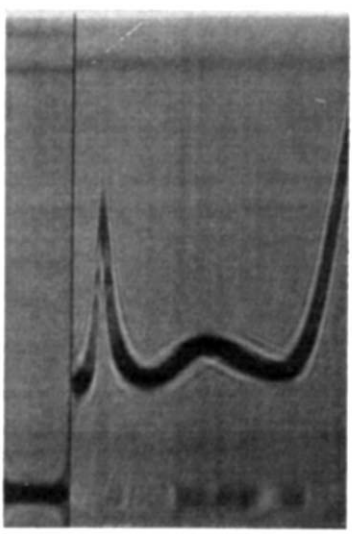

Fig. 4. Sedimentation of PVPB in water using synthetic boundary cell. Left hand boundary between zero and 1.0 %, right hand boundary between 1.0 and 2.0 % solutions.

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In order to verify the reasoning given here for expecting a spreading boundary between 2 solutions of a macromolecule having similar values of S_{20}, a study was made of the boundaries formed under similar conditions by the polyelectrolytes, PVPB and PMA. Synthetic boundaries were formed between 2 % and 1 %, and between 0.5 % and 0.25 % solutions respectively in water as solvent. These concentrations were chosen since the change in the value of the sedimentation coefficient between zero and 1 % concn. for PVPB or zero and 0.25 % for PMA is very much greater than that between 1 and 2 % for PVPB or 0.25 and 0.5 % for PMA. It was found, as is shown in Fig. 4 for PVPB, that in both cases the boundary between zero and finite concentration was much sharper than that between the 2 finite concentrations. This result shows that for PMA and PVPB, the sharpness of the boundary depends on the relative sedimentation coefficients above and below the boundary. The normal theory of boundary sharpening based upon the concentration dependence of the sedimentation coefficient may thus be applied. DNA preparations are known to be polydisperse with respect to sedimentation coefficient from the measurements of SHOOTER AND BUTLER[12] and of SCHUMAKER AND SCHACHMAN[13] at very low concentrations, yet as the sedimentation boundary does not spread under conditions where the sharpening effect due to concentration dependence does not operate, it is necessary to postulate a self-sharpening effect of a new type. The JOHNSTON-OGSTON effect will not explain the results as it cannot operate under conditions where there is no great difference in the sedimentation rate across the boundary.

Some guidance concerning the nature of other boundary sharpening effects has been obtained from a study of the sedimentation of DNA in ethyl alcohol solutions[14]. Solutions of DNA in ethyl alcohol have a sufficiently low electrical conductivity[15] to suggest that under these conditions DNA is behaving as a non-electrolyte. The sedimentation coefficient between 0.2 and 0.8 % shows extreme concentration dependence but the DNA nevertheless forms a spreading boundary. Comparison of the sedimentation behaviour of DNA in water and alcohol solutions shows that in both solvents the sedimentation coefficient is very dependent on concentration, but whereas the boundary in water as solvent is hypersharp, that in alcohol spreads. The only known difference between the DNA in the 2 solvents is that in water it is charged and behaves as an electrolyte, whereas in alcohol it behaves as a non-electrolyte. It would therefore appear reasonable to assume that the formation of the hypersharp boundary is related to the charged nature of the DNA molecule in aqueous solution. It is unlikely that the charge alone is responsible for the hypersharp boundary since other polyelectrolytes, such as PMA, show spreading boundaries. A factor that may, however, affect boundary behaviour, is the concentration dependence of charge. The variation of the charge on the DNA and the PMA ion with change of concentration of the macro-ion and also of added electrolyte has been determined by GOODEN, INMAN AND JORDAN[16]. Their data show that at concns. of DNA above about 0.05 % in the absence of sodium chloride, there is little variation of the charge with concentration, whereas below this concentration a strong concentration dependence is observed. If variation of the charge on the macro-ion across a sedimenting boundary, due to the concentration gradient, is responsible for the hypersharpness, then a synthetic boundary formed between solutions of 0.05 and 0.1 % DNA in the absence of sodium chloride should not be hypersharp, but should give a spreading boundary. This prediction is in agreement with experiment as shown in Fig. 5. Furthermore, the

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data for the charge on the PMA ion in the absence of added electrolyte are consistent with the charge variation hypothesis since in the experiments described above, a spreading boundary was observed between 0.25 and 0.5 % PMA solutions and the variation of the charge over this concentration range is very small[16].

Addition of sodium chloride to solutions of DNA increases the charge on the macro-ion and displaces the region of rapid variation of charge to higher concentrations. Thus whereas in the absence of sodium chloride the variation of charge between 0.1 and 0.05 % solutions of DNA is small, in 0.01 *M* sodium chloride the variation of charge over this concentration range is considerable[16]. Comparison of the synthetic boundary diagrams for this concentration range in water and in 0.01 *M* sodium chloride (Figs. 5 and 6) shows that the addition of sodium chloride had caused a sharpening of the synthetic boundary in agreement with increased concentration dependence of charge.

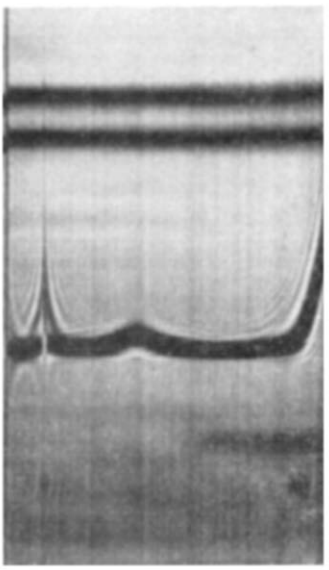

Fig. 5. Sedimentation of DNA in water using synthetic boundary cell. Left hand boundary between zero and 0.05 %, right hand boundary between 0.05 and 0.10 % solutions.

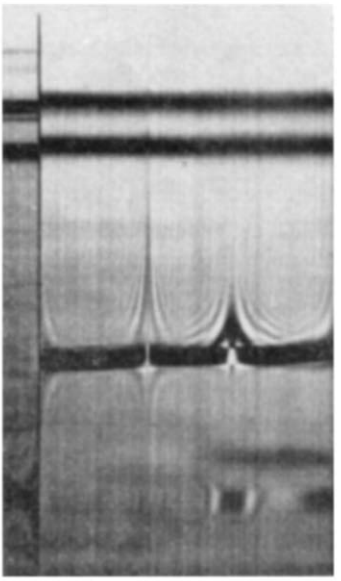

Fig. 6. Sedimentation of DNA in 0.01 *M* NaCl using synthetic boundary cell. Left hand boundary between zero and 0.05 %, right hand boundary between 0.05 and 0.10 % solutions.

It is thus to be concluded that the boundary sharpening effect in DNA solutions is probably related to the change in the charge of the sedimenting macro-ions across the boundary. A theoretical description of this phenomenon has yet to be obtained. SVEDBERG's primary salt effect[17] does not describe the behaviour since this does not operate at high salt concentrations where hypersharp boundaries are still observed. Moreover, it predicts that in the absence of added salt, the macro-ion with highest effective charge in the low concentration region of the boundary will sediment more slowly (because of the large number of counter ions) than those in the higher concentration region; this would give rise to an increase in the spread of the boundary.

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ON THE PIGMENT SYSTEM OF THE RED ALGA *PORPHYRA LACINEATA*

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SUMMARY

Absorption and fluorescence around 725 mμ of suspensions of the red alga *Porphyra lacineata* were studied both with and without the phycobilins attached to the pigment system.

The *in vivo* occurrence of 2 chlorophyll *a* types was confirmed spectroscopically. Their red absorption maxima are located at 678 and 672 mμ respectively. Removal of the phycobilins did not affect the shape of the red absorption band of these types. It is therefore concluded that phycobilins are not responsible, *e.g.* by complex formation, for the occurrence of one or both of these types. It is possible that withdrawal of the phycobilins causes quenching of chlorophyll fluorescence. The "pigment X" fluorescence around 725 mμ remains unaffected by removal of the phycobilins.

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