THE EFFECT OF HEAT AND X-RAYS ON DEOXYRIBONUCLEIC ACID

by

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The effect of heat on the physical properties of DNA solutions has been studied by several workers[1], who have found that after heating dilute solutions at temperatures of about 80° C or above the viscosity is greatly reduced. Different findings have been reported as to the effects of this treatment on the sedimentation behaviour. DEKKER AND SCHACHMAN[2] found that after heating a 0.005% solution of DNA for 15 min at 100° the sedimentation coefficient was reduced from $S = 20$ to $S = 6$, which they interpreted as indicating a decrease of molecular weight. They therefore proposed a model of DNA in which the twin helices of WATSON AND CRICK[3] were interrupted by breaks at different points as a result of which the whole structure breaks on heating into fragments of low molecular weight. On the other hand DOTY AND RICE[4] report that a similar treatment increased the sedimentation constant extrapolated to infinite dilution from $S = 20$ to $S = 30$ and that the molecular weight determined by light scattering remained unchanged, although the radius of gyration was greatly reduced.

In an attempt to resolve these discrepancies, we have determined the effect of heat on the sedimentation coefficients and viscosities of DNA which has been treated in various ways. The measurements were made at very low concentrations at which it has been shown that heterogeneity with respect to sedimentation coefficient is observed[5]. The observations therefore give not only a measure of the effect of heating on the mean sedimentation coefficient but also a measure of its effect on the distribution of sedimentation coefficients.

EXPERIMENTAL

Viscosity

The reduced specific viscosities were determined at 25° in the Couette viscometer at such low concentrations that further dilution in 0.2 M sodium chloride has no effect. The measurements also refer to zero shear rate. The figures given are therefore identical with the intrinsic viscosity of the material at zero shear rate under the conditions stated. Different samples of TNA 23 differed appreciably in their intrinsic viscosities, but heating produced similar effects.

Sedimentation

The sedimentation curves were obtained using the Spinco ultra-centrifuge[5]. The experimental details and validity of these curves will be discussed elsewhere. The curves shown in the figures are the integral distribution curves *e.g.* at a sedimentation coefficient of $S = 20$ the height of the curve gives the total fraction of the DNA which has sedimentation coefficients of 20 or less.

References p. 502.

RESULTS

Some typical sedimentation coefficient distribution curves obtained before and after heating 0.005% solutions of DNA in 0.2*M* NaCl are shown in Fig. 1. Table I gives the average values of the sedimentation coefficients and the intrinsic viscosities, and also the result obtained after heating a solution of DNA in 0.2*M* NaCl at a concentration of 0.05% and then diluting to 0.005%.

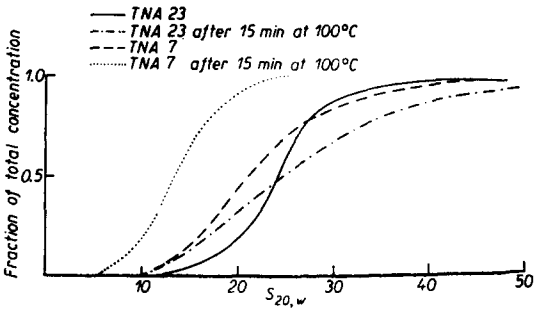

Fig. 1. Effect of heat on sedimentation coefficient distribution of two samples of DNA. 0.005% DNA in 0.2 *M* NaCl.

It can be seen that in all cases the effect of heating is to reduce the intrinsic viscosity but the effect on sedimentation behaviour of the various samples differs to some extent. In two cases III and IV the average sedimentation coefficients are unaffected by heating, but the spread of sedimentation coefficients is changed, there being an increase of material with both high and low sedimentation coefficients. In two other cases I and II the whole distribution curve is moved to lower values of sedimentation coefficient. It may be significant that the two latter preparations were prepared by an enzyme method[6a] involving incubation with chymotrypsin and the former pair were prepared by detergent methods[6b]. It is clear from V however that the effect of heat depends to some extent on the concentration at which the DNA solution is heated. If the susceptibility to heat is due to breaks in the nucleotide chains as suggested by DEKKER AND SCHACHMAN, it should be possible to produce an increased susceptibility to

TABLE I

EFFECT OF HEATING (100° for 15 mins) ON DILUTE DNA SOLUTIONS

Preparation	*Concentration* %	*Wt. average sed. coefficient* ($S \cdot 10^{13}$)		*Specific reduced viscosity in 0.2 N NaCl*	
		Before heating (in salt)	*After heating*	*Before heating*	*After heating*
I. TNA 7	0.01	20	14.4	—	—
	0.005	23.4	15.8	27	1.7
II. TNA 15	0.01	19.6	15.2	52	3
III. TNA 23	0.01	20.0			
	0.005	24.0	24.0	50	1
TNA 23*	0.01	—	—	273***	7***
IV. DNA-SB-11	0.01	20.8	20.0	72	4.3
V. TNA 23**	0.005	27	36	80	5

* Heated in water only, not in 0.2 *N* NaCl.
** Heated in 0.05% solution.
*** Measured in water.

References p. 502.

TABLE II

EFFECT OF X-RAYS ON HEAT STABILITY OF DNA PREPARATION (DNA 23)

	Mean sedimentation coefficient $\times 10^{13}$		*Mean sedimentation coefficient after 15 min 100°* $\times 10^{13}$	
	0.01%	0.005%	0.01%	0.005%
Original solution				
irradiated 9,000 r	20	24	—	24
4 h after X-rays	16.8	20		
24–26 h after X-rays	13.8	20		
14 days after X-rays	16	19	10	12
Solid irradiated				
10^5 r	—	23	—	20
10^6 r	—	28	—	17

heat *e.g.* by the action of X-rays, which have been shown among other effects to cause breaks in the nucleotide chain[7]. That this is the case is shown by Table II which gives the mean sedimentation coefficients of DNA 23 after treatment in solution with X-rays (9000r). The solutions were given a considerable time for the completion of "after-effects" of the irradiation and were then heated. It can be seen that the effect of heat is markedly increased by this treatment.

Experiments were also made to determine whether similar damage was caused by the irradiation of DNA in the solid state. The irradiation was effected by 1.2 million volt electrons from the Van de Graaff apparatus. The DNA (TNA 23) was not specially dried and contained about 20% of moisture. Nevertheless its sensitivity to irradiation is much less than in solution. Doses of between 10^5r and 10^6r cause only a small change of viscosity, as determined in Frampton viscometers. At doses to $5 \cdot 10^5$r the mean sedimentation coefficient is not much affected, but there is a marked increase in the spread of the distribution curves resulting from an increase both of material with low and high sedimentation constants *i.e.* a combination of degradation and "aggregation" occurs. At 10^6r aggregation predominates. In all these cases heating lowers the mean sedimentation coefficient to a greater extent than in the original material and the effect is greater the greater the dose of radiation (see Fig. 2 and Table II).

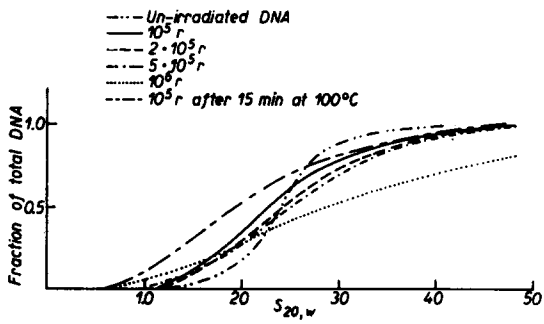

Fig. 2. Effect of irradiation of solid DNA on sedimentation coefficient distribution. 0.005% DNA in 0.2 *M* NaCl.

It is possible that the dodecyl sulphate used in the preparation of TNA 23 and probably still present in small amounts has a stabilizing effect. To test this we have added dodecyl sulphate (weight equal to that of DNA) to a solution of a DNA prepared by the enzyme method (TNA 15) and we have found that the stability to heating was not appreciably affected. Any detergent that is present does not therefore appear to mask weak points in the DNA structure.

References p. 502.

Another possibility is that the behaviour is influenced by the residual amounts of protein remaining in the DNA preparations. It has in fact been shown that the effect of heat on nucleoprotein preparations is to a great extent determined by the protein content of the material[8], and that when more than 17% of protein is present the viscosity is increased by heating. In order to test whether any affects of this kind occur when small amounts of protein are present, we added histone to a solution of TNA 23 (approx. 1% of the weight of DNA) but no change of the sedimentation constants or viscosity before or after heating were observed (Fig. 3).

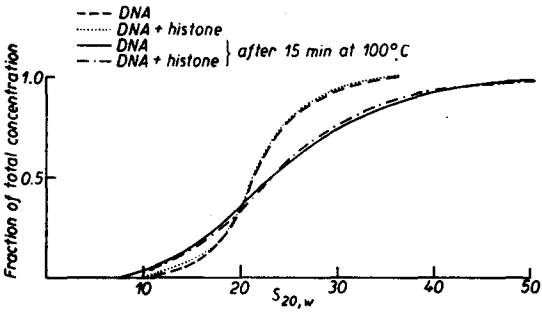

Fig. 3. Effect of addition of histone on sedimentation coefficient distribution of DNA. 0.005% DNA in 0.2 *M* NaCl.

DISCUSSION

An estimate of the molecular weight is obtained from the equation of MANDELKERN *et al.*[9]

$$\frac{S_0 [\eta]^{1/3}}{M^{1/3}} = \frac{K (1 - \bar{V}\varrho)}{\eta_0 N}$$

where S_0 is the sedimentation constant at infinite dilution, $[\eta]$ the intrinsic viscosity, and M the molecular weight. K is a constant which is $2.5 \cdot 10^6$ for a flexible coil and varies between 2.2 and $3.6 \cdot 10^6$ for ellipsoids of increasing axial ratios. We have used the value $2.5 \cdot 10^6$ in the calculations. The values of S_0 for the normal solutions have been determined by extrapolation of the mean values of S obtained at concentrations in the range 0.02–0.005%. The extrapolation to zero concentration gives S_0 with an accuracy of $\pm$ 10%. With the exception of TNA 7 for which we have results at 0.01% and 0.005% the sedimentation experiments with the heated solutions were confined to one concentration. For the purpose of calculation, we have assumed that the change in S_0 is proportional to the change of S observed at the concentration used, *i.e.* that S_0 for TNA 15 is reduced on heating by 23%, while S_0 for TNA 23 and DNA-SB-11 is unaffected. Even in those cases where there is little change of the mean sedimentation constant the apparent molecular weight is reduced. It must be remembered however that the intrinsic viscosity is not necessarily a weight average property of the solute and since changes in the size and shape distribution are produced on heating the molecular weights before and after heating (Table III) may not refer to the same weighted average. For this reason a precise comparison of the effects of heating the various samples cannot be made.

The general picture of the effect of heating DNA which is consistent with these observations is shown in Fig. 4. Initially the individual strands of the twin helix of DNA are presumed to be broken at different points along their length. On heating some of the hydrogen bonds holding the two nucleotide chains together are broken allowing the single strands to fray out from the main structure as in Fig. 4a. The

References p. 502.

TABLE III

Sample	Before heating			After 15 mins at 100° C		
	$S_0 \times 10^{13}$	$[\eta]$	$M \times 10^{-6}$	$S_0 \times 10^{13}$	$[\eta]$	$M \times 10^{-6}$
TNA 7	22 ± 2	27	6.6 ± 0.9	17	1.7	1.1
15	24 ± 2	52	10.5 ± 1.3	19	3	1.8
23	26 ± 2	50	11.6 ± 1.4	26	1	1.6
23 (9000 r)	16.5	24	4	9	2	0.5
DNA-SB-11	20	72	9.4	20	4.3	2.3

structure collapses about the more flexible parts of the molecule joined by single strands of nucleotide chain as in Fig. 4b. This reduction in the asymmetry of the molecule is accompanied by a decrease in frictional resistance which will reduce the viscosity but will also lead to a rise in the rate of sedimentation. If, however, some of the breaks in neighbouring nucleotide chains are sufficiently close together then the molecule will break up into smaller units as shown in Fig. 4c. The change observed in the sedimentation coefficient distribution of TNA 23 on heating can be accounted for in terms of a combination of effects *b* and *c* above. It may be observed however that if the heating is done in more concentrated solutions it is quite possible that other effects will occur, *e.g.* aggregation may occur by the formation of hydrogen bonds between "frayed out" segments of different particles. Under these circumstances the increase of molecular weight due to aggregation might well balance the decrease arising from the degradation process illustrated in Fig. 4c. This latter effect is illustrated in Table I, V. The varied findings of different investigators may well be due to effects of this kind, if heating is carried out at different concentrations.

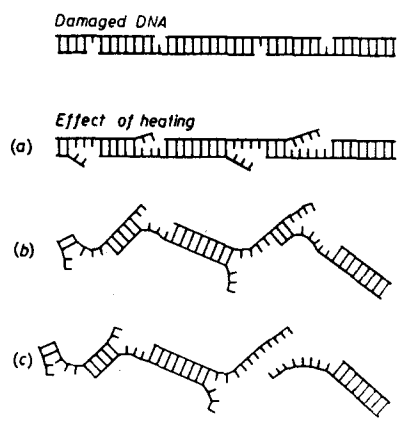

Fig. 4. Suggested effects of heating on DNA.

The results given suggest that in all cases the samples of DNA are degraded on heating but that the extent of the degradation and the distribution of size and shape of the fragments produced depends upon the way in which the DNA has been prepared. The study of the DNA damaged by X-rays suggests that susceptibility to heat is associated with the presence of breaks or weak points in the single polynucleotide strands. While it is possible that such weak points exist in native DNA the experiments do not preclude the possibility that they are artefacts produced during preparation.

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References p. 502

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SUMMARY

The effect of heating solutions of deoxyribonucleic acid on the sedimentation coefficient distribution and viscosity has been observed. There is in general a decrease in molecular weight, but the mode of preparation of the samples influences to some extent the degree of degradation and the distribution of size and shape of the fragments produced. Since an increased susceptibility to heat can be induced by X-irradiation of the solutions, the findings are in accordance with the view that degradation results from the presence of breaks in the polynucleotide strands.

When DNA is irradiated in the solid state much higher doses are required to produce changes comparable with those obtained by irradiating dilute solutions, and a dose of 10^6r gives rise to marked aggregation.

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