The u.v.-absorption of calf-thymus DNA

It is generally accepted that the extinction coefficient of DNA measured at 2590 Å is dependent on the degree of denaturation of a DNA solution. This is a consequence of the hyperchromicity displayed by native DNA on denaturation. The atomic extinction coefficient with respect to phosphorus of DNA prepared by mild methods is in the vicinity of 6600 (see ref. 1); however, many of the reported values lie in a broad range about this figure. Extinction coefficients as low as 5900 (see ref. 2), and 6000 (see ref. 3) and as high as 7500 (see ref. 4, 5) have been reported for native DNA. The values obtained by other investigators[6–10] fall within the region 6300–6600. In an attempt to determine a more accurate extinction coefficient for native DNA nine samples of calf-thymus DNA were prepared under slightly different conditions, the various modifications being designed mainly to test the effect of ionic history. Samples which were never subjected to low ionic strengths during preparation yielded an ε(P) of 6540, while samples dissolved in water during preparation gave a value of 6660.

In a physico-chemical study of the denaturation of aqueous DNA solutions, to be submitted for publication in this journal, it was desirable to prepare samples of DNA free from NaCl, and, moreover, it was essential in the preparation to use aqueous solutions without added salt. To fulfil these conditions it was necessary to carry out the final precipitation with ethanol from a salt-free solution rather than from 1 M NaCl as commonly employed. The u.v.-absorption of various samples subjected to this treatment was compared with measurements made on DNA samples that had never been dissolved in water of low ionic strength. Table I lists the preparative histories of the nine samples used in this and later investigations.

The u.v.-measurements were made on solutions of DNA in 0.1 M NaCl prepared in one of two ways: (a) DNA was dissolved in water, at 4°, to give a concentration of $3 \cdot 10^{-3}$ M with respect to phosphorus and subsequently made 0.1 M with respect to NaCl by the addition of concentrated salt solution and then diluted to $5 \cdot 10^{-5}$ M DNA with 0.1 M NaCl; (b) DNA was dissolved directly in 0.1 M NaCl and later diluted with 0.1 M NaCl to $5 \cdot 10^{-5}$ M DNA. All ε(P) values refer to the phosphorus concentration determined on each stock solution.

As no significant difference was noticed between methods (a) and (b) for the samples that had been dissolved in water of low ionic strength, these results were pooled and are shown in Table II, about equal numbers of both samples being used. The extinction coefficient of the samples dissolved in water during preparation was found to be 6660 $\pm$ 30. The results for the DNA samples never dissolved at low ionic strength during preparation are given in Table III. It can be seen that a small but significant difference between methods (a) and (b) was found. If the sample was dissolved in water before adjusting to 0.1 M NaCl a value of 6630 was obtained in agreement with the results expected from Table II; however, if the sample was dissolved directly in 0.1 M NaCl an average value of 6540 was obtained. This value is to be regarded as the extinction coefficient of native DNA as prepared by the detergent method[11] and is very close to the value of 6650 $\pm$ 50 recorded by CHARGAFF AND LIPSHITZ[12], although these measurements were made before the possibility of denaturation of DNA by exposure to solutions of low ionic strengths was fully

Abbreviation: DNA, deoxyribonucleic acid.

TABLE I

PREPARATIVE HISTORIES OF NINE SAMPLES OF CALF-THYMUS DNA

Preparative history	*Dissolutions in water*
Preparation 1 Prepared by the detergent method[11] except that the final fibres were washed with ethanol only. Dried over P_2O_5 for 2 days *in vacuo*.	2
Preparation 2 As for 1, except that the final precipitation was carried out from aqueous solution. Dried over P_2O_5.	2
Preparation 3 As for 2, except that the DNA redissolved in water and precipitated from saline solution three times then redissolved in water and precipitated in the absence of salt. Dried over P_2O_5.	4
Preparation 4 Portion of 3 dissolved and precipitated from water.	5
Preparations 5 and 6 As for 1, except that 0.1 *M* NaCl used instead of water at all stages. Air dried.	0
Preparation 7 Prepared by the method of GULLAND *et al.*[13] and finally dissolved and precipitated from water.	2
Preparations 8 and 9 As for 1, except that 0.0014 *M* NaCl was used instead of water at all stages. Air dried.	0

TABLE II

ATOMIC EXTINCTION COEFFICIENTS MEASURED AT 2590 Å OF DNA SAMPLES DISSOLVED IN WATER DURING PREPARATION

Preparation	*Number of measurements*	*ε(P) Pooled results of methods (a) and (b)*	*Standard error*
1	14	6614	26
2	26	6646	23
3	18	6651	20
4	44	6705	12
7	25	6613	22
	Average	6657	

appreciated. It can be concluded that dissolution of native DNA in water at a concentration of $3 \cdot 10^{-3}$ *M* results in an irreversible increase in extinction coefficient of 1.4%, which is compatible with a small amount of denaturation. Unfortunately this small amount of denaturation will complicate any study involving aqueous solutions of low ionic strength.

The possible denaturing effect of precipitation with ethanol, which might be present in all the results so far given, was checked as follows. The ε(P) was determined for a solution of preparation 6 before precipitation with ethanol after the first deproteinisation, and corrected for the residual detergent present. The value was found

TABLE III

ATOMIC EXTINCTION COEFFICIENTS MEASURED AT 2590 Å OF DNA SAMPLES NEVER DISSOLVED IN WATER OF LOW IONIC STRENGTH DURING PREPARATION

Preparation	$\varepsilon(P)$	
	Method (a)	*Method (b)*
5	6540	6570
6	6690	6530
8	6670	6560
9	6620	6490
Average	6630	6540

to lie between 6580 and 6630 and was not significantly different from the overall mean value.

A source of error in the measurement of extinction coefficients in the case of DNA is apparent on inspection of the water content of solid samples. For the preparations initially dried over P_2O_5 the water content displayed a general rise from 5–12 % up to 20–30 % on exposure to atmospheric humidity. In all the preparations studied, however, there was never any predictable change in water content except for the general rise noted above. Because of the variation in water content any absorptivity measurements which ultimately depend on the weighing out of DNA will be inviting error. This could come about, for instance, if the phosphorus content of a particular preparation was determined and at a later date the u.v.-measurements made on a different weighed DNA sample.

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