Spectrophotometric Studies on Aggregation of Denatured DNA

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Spectrophotometric studies have shown that aggregation of denatured DNA is dependent upon temperature (with an optimum at thirty seven degrees), concentration of the macromolecule, and on solvent conditions. The aggregates dissolve at very low (0 °C) and high temperatures (60 °C), and in very low concentrations of either macromolecule or salt in the solvent.

It is well known that some aggregation most likely takes place during any renaturation, this can be eliminated or reduced to a great extent by enzymes that digest denatured DNA specifically or by exposing renatured DNA to temperatures near the Tm, or at very low temperatures in solvents of low ionic strength. It has been recently shown by MAY [2] that one can study the formation of aggregates of denatured DNA by rheological technique. Such treatment has been found not to provoke aggregation at either low (T ≳ 0.3 °C) or high (T ≳ 70 °C) temperatures, the most favourable temperature for aggregation being about 40 °C. According to this worker aggregates would continue to form in solution even at rest. Presumably such aggregation at constant temperature would proceed slowly even when the shearing stress is removed. The object of the present experiments is to study this phenomenon at various conditions with the help of spectrophotometry. The results of denaturation at different temperatures have been presented in an earlier communication [3]. Optically, however, the effects of aggregation could hardly be distinguished from those of renaturation. So in most of the experiments presented here calf thymus DNA has been used. Results of other authors indicate that this DNA is less renaturable because of its less GC content [1-4]. Some experiments have also been repeated with E. coli B DNA. The criteria for detecting aggregation is the fall in absorption at the two maxima of DNA absorption spectrum in the ultraviolet region, namely, one at 258 mμ (X) and the other at 204 mμ (Y) [5,6]. Absorption studies at the 204 mμ peak have been made with special care for the effect due to stray light, salt absorption, instrumental cut-off and macromolecular scattering in this region. A detailed study concerning the above factors would be found in earlier papers by the present worker [5,6]. A

2 P. May, J. molecular Biol. 9, 263 [1964].
5 S. Basu, Biopolymers 3, 876 [1967].
SPECTROPHOTOMETRIC STUDIES ON AGGREGATION OF DENATURED DNA

P.M.Q. II spectrophotometer was used for absorption studies. Temperatures were maintained constant in thermostatic baths provided with German platinum contact thermometer (± 0.01 °C). The gradual decay in absorbance (after complete denaturation) of denatured DNA relative to the starting denatured material has been represented graphically as a function of time. By comparing curves obtained under different experimental conditions, the influence of various factors affecting the formation of aggregates has been determined.

The calf thymus DNA is a gift sample from Prof. S. Zamennon (California). DNA from E. coli B has been extracted in highly polymerised form by the method of Marmur. Pronase (CalBioChem) has been used to remove proteins and heat treated RNase (Sigma Chem) to remove RNA. The characteristics of the calf thymus DNA are N: 13.25%, P: 7.84%, N/P: 1.69%, H₂O: 10.52%. DNA of various concentrations (10 – 50 μg/ml) in BPES (pH 7.00) at different ionic strengths (0.2 – 0.0002 M Na⁺) has been denatured completely by keeping at various constant temperatures (60 °C – 0 °C). Denaturation time is different at different temperatures ranging from a few hours to a few days. Complete denaturation of DNA has been assured by nearly 40% saturation hyperchromicity at 258 μm peak and 80% at 204 μm peak at the end of respective periods. In order to detect any enzymatic or non-enzymatic actions during storage three techniques namely, electron microscopy, sedimentation and the method of acid (TCA) precipitation have been followed. These techniques have indicated an identity between DNA denatured by conventional melting and the material which has been kept for various periods at temperatures considerably below the Tₘ, a treatment which has been hitherto thought not to cause any significant structural changes of DNA.

For the present investigation, samples showing full denaturation (i.e. hyperchromicity) are merely held at their respective incubation temperatures for various durations, and absorbances of these samples relative to the starting denatured material are recorded with time at room temperature. The denatured samples on standing show gradual decrease in absorbance.

In order to find the effect of formaldehyde a few samples before heat treatments are mixed with 1% formaldehyde (neutralised and acid freed according to Freifelder and Davison). The results of absorption studies on calf thymus DNA only are shown in Figs. 1 – 3. The data for E. coli B DNA have been omitted since the results are qualitatively similar.

![Fig. 1. Decrease in relative absorbance at 258 mμ (X) and 204 mμ (Y) with time at different temperatures (0°, 14°, 37° and 60 °C) for a completely denatured calf thymus DNA at a concentration of 10 μg/ml in BPES (0.02 M Na⁺).](image1)

![Fig. 2. Decrease in relative absorbance at 258 mμ (X) and 204 mμ (Y) with time for a completely denatured calf thymus DNA at concentrations of 10 and 50 μg/ml. Solvent: BPES (0.002 M Na⁺), temperature: 14 °C.](image2)

**Influence of temperature**

The plots in Fig. 1 show that diminution in absorption of calf thymus DNA at the absorption maxima is completely absent for the lowest temperature (0 °C) and its rate increases with the rise of temperature.

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9 D. Freifelder and P. F. Davison, Biophys. J. 3, 49 [1963].
Fig. 3. Decrease in relative absorbance at 258 m\(\mu\) (X) and 204 m\(\mu\) (Y) with time at different ionic strengths for a completely denatured calf thymus DNA of concentration 50 \(\mu\)g/ml in BPES (0.002 M, 0.0002 M Na\(^+\)); temperature: 14 °C.

being maximum near 37 °C which is about \((T_m - 25)°C\) and then falls, although the temperature is increased to 60 °C.

**Influence of nucleotide concentration**

Fig. 2 shows that decrease in absorbance at the DNA maxima is encouraged at higher DNA concentration (50 \(\mu\)g/ml). At low DNA concentration (10 \(\mu\)g/ml) the effect is leveled off after a certain time. The incubation temperature (14 °C) chosen was roughly 25 °C below the \(T_m\) of DNA (calf thymus) is solvent 0.002 M BPES.

**Influence of ionic strength**

Fig. 3 shows that the rate of decrease in absorbance of the same DNA at 14 °C is higher at higher ionic solvent and vice-versa.

**Influence of formaldehyde**

Formaldehyde preventing renaturation of denatured units has been next used\(^{10}\). It has been observed that a DNA (either calf thymus or \(E.\ coli\) B) solution (50 \(\mu\)g/ml, 0.2 M Na\(^+\)) heated for 10 min at 100 °C and then rapidly chilled or annealed in the presence of 1% formaldehyde exhibits no decrease in absorption for many days. Similar results have been obtained when the same DNAs denatured by storage were kept at various temperatures in the presence of the reagent. On the contrary, under similar treatments over the same durations, some loss in absorbance occur in the absence of the reagent. Some of the above experiments have also been performed in \(E.\ coli\) B DNA denatured under identical conditions of incubation.

From the present state of knowledge the time dependent changes observed in the DNA absorption maxima after complete denaturation under different conditions could be attributed to any one or a number of causes e.g. renaturation, formation of complexes that occur between pairs of molecules (i.e. aggregation or association) or between solute and solvent (e.g. H-bonding), loss of DNA from the solution by adsorption or precipitation. If the decrease in absorbance of denatured DNA solution is attributed to adsorption which is a surface phenomenon, the same effect should occur to whatever extent for native solution. But results obtained are quite different, i.e. native DNA never shows loss of material due to interface adsorption. Also, both adsorption and precipitation are always more the lower the temperature, and are not favoured at any particular temperature (viz. 37 °C). The plausible phenomena showing simultaneous dependence on critical temperature and concentration of macromolecules are renaturation and aggregation\(^{9}\).

Presumably renaturation can be used only when speaking about time-dependent absorbance changes of bacterial DNA and aggregation can occur in both bacterial and mammalian cases. The present studies have shown that the final decrease in absorbance are qualitatively similar for both the DNAs. However, for \(E.\ coli\) B DNA the two effects i.e. renaturation and aggregation have been distinguished for the initial period of incubation (or annealing) by the measurement of absorbances. These results are not shown here. For the first few hours (4 hr at 37 °C) samples of specific concentrations showing recovery of absorbance after annealing have shown adherence to LAMBERT-BEER's Law on dilution, while after this initial period such measurements carried out on aged samples have indicated large deviation from this law. Both native and denatured DNA have been reported to show absorbance-concentration dependence\(^{5,6,11}\). Hence it is expected that renatured DNA would also obey this law, while the denatured aggregated complexes would not. In the case of experiments done on calf thymus DNA the time dependent changes have been generally referred mostly as aggregation\(^{6}\). In this case, however, the decrease


in absorbance of denatured DNA by a factor of 5 to 10 fold (Figs. 1–3) could not be all due to helical or nonhelical aggregation. This leads one to suspect that the changes in absorbance of solutions on long standing were partly due to gradual formation of complexes between the polymeric molecules and solvent e.g. H-bonding and further condensation of these aggregated complexes causing shielding of chromophores which added more to deviation from Lambert-Beer’s Law.

We summarise and explain our observations in these lines.

(i) The formation of DNA aggregates irrespective of the type of DNA is optimum near 37 °C in 0.02 M BPES.

(ii) Disaggregation is provoked at very low (0 °C) and high temperatures (60 °C), at very low nucleate and salt concentrations.

(iii) Formaldehyde prevents renaturation as well as aggregation of denatured molecules.

These observations are similar to those of May who concluded that to obtain maximum amount of aggregation denatured DNA should be incubated at a optimum temperature nearly 25 °C below the melting temperature. In the present case, the melting temperatures for calf thymus and E. coli DNA in 0.02 M BPES buffer were roughly 62.5 °C and 68 °C respectively. So the temperature i.e. 37 °C showing maximum aggregation for these DNAs in the above solvent corresponds well to the above conclusion of May. The observed results showing dependence of aggregation upon temperature, concentration of the macromolecule and on solvent condition are also in general agreement with the theoretical analysis of helical and non-helical aggregations of macromolecules by Oasawa and Kasai.

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12 F. Oasawa and M. Kasai, J. molecular Biol. 4, 10 [1962].