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Structure of the Replicative Form of Bacteriophage ϕX174

VI.† Studies on Alkali-denatured Double-stranded ϕX DNA

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Double-stranded ϕX DNA which accumulates after infection with bacteriophage ϕX174 in the presence of chloramphenicol consists mainly of twisted circular double-stranded DNA with no single-strand breaks (component I) and of circular double-stranded DNA, in which single-strand breaks are present (component II). Component II in M-NaCl is denatured with alkali within a sharp pH range, but component I is denatured over a wide pH range (pH 11·2 to 12·4). The midpoints of the transition in M-NaCl are pH 11·3 and 11·9, respectively. Component I in 0·1 M-phosphate is irreversibly denatured at pH 12·7 to 12·8 or higher, giving rise to a structure which sediments in neutral M-NaCl with an $S_{20,w} = 40$ S. The configuration of denatured double-stranded ϕX DNA has been studied by means of velocity sedimentation, isopycnic centrifugation in CsCl and spectrophotometry, and also the reactivity for formaldehyde has been determined. From the results it is concluded that denatured double-stranded ϕX DNA occurs as a highly twisted structure in which all or almost all hydrogen bonds have been disrupted. Denatured double-stranded ϕX DNA does not renature at high temperatures in low or high salt concentrations, but is converted spontaneously to native double-stranded DNA after introduction of a single-strand break by pancreatic DNase. The implications of these findings for the structure of denatured double-stranded DNA are discussed.

1. Introduction

Double-stranded ϕX DNA is formed after infection of *Escherichia coli* C with bacteriophage ϕX174 (Sinsheimer, Starman, Nagler & Guthrie, 1962), which harbours a single-stranded circular DNA molecule (Sinsheimer, 1959). The double-stranded form of ϕX DNA has been shown to occur in three different structures. Double-stranded phage DNA which is isolated from bacteria infected in the presence of chloramphenicol has a circular structure with two separately continuous strands (component I). Besides the main component a minor component has also been found with a circular structure in which single-strand scissions are present (component II)

† Paper V in this series is Jansz, Baas, Pouwels, van Bruggen & Oldenziel (1968).

(Jansz & Pouwels, 1965). Occasionally also a third component (component III) is observed (Jaenisch, Hofschneider & Preuss, 1966) which is linear and probably arises from component I or II by a double-strand cleavage.

Components I and II show a difference in sedimentation behaviour which is explained at present by a difference in conformation rather than in molecular weight. Component I would be a circular molecule in which tertiary twists are superimposed on the ring structure, whereas component II would have an extended ring structure in which no such additional twists are present (Burton & Sinsheimer, 1965; Pouwels, Jansz, van Rotterdam & Cohen, 1966; Roth & Hayashi, 1966). Component I may be converted into component II by introduction of a single break in one of the two strands by means of pancreatic DNase (Jansz & Pouwels, 1965; Jansz, Baas, Pouwels, van Bruggen & Oldenziel, 1968).

Linear double-stranded DNA molecules isolated from bacteria or bacteriophages will become single stranded upon heating or at extreme values of pH. The two strands of a circular double-stranded DNA molecule, like the twisted form of various viral DNA's (Weil & Vinograd, 1963; Pouwels & Jansz, 1964; Crawford & Black, 1964; Bode & Kaiser, 1965), cannot be separated, however, since the two strands are separate but continuous. The topological restraint that is imparted to the molecule by the continuity of the two strands is revealed upon denaturation of the DNA molecules. This restraint, however, is lost after introduction in the circular DNA molecules of a single-strand scission: component I shows a high resistance towards heat denaturation, in contrast to component II and probably also component III. It is the purpose of the present paper to describe experiments on the alkali denaturation of components I and II, which show that these components also differ greatly in sensitivity for alkali denaturation.

After irreversible denaturation of component I with alkali, a structure is formed which sediments about twice as fast in M-NaCl (pH 7) as before denaturation. Physicochemical evidence is presented in this paper which suggests that the alkali-denatured double-stranded ϕX DNA is a fully denatured molecule which will not renature unless a single-strand break is introduced into the molecule.

2. Materials and Methods

(a) *Preparation of double-stranded ϕX DNA*

Double-stranded ϕX DNA is prepared from *E. coli* C infected with phage as described previously (Jansz, Pouwels & Schiphorst, 1966). Double-stranded ϕX DNA labelled with ^{32}P is prepared from cells infected in the presence of ^{32}P-labelled inorganic phosphate (0·6 mc/mg P). The remainder of the isolation procedure is the same as for non-labelled double-stranded ϕX DNA.

(b) *Sedimentation analysis*

Velocity sedimentation was performed in a Spinco model E analytical ultracentrifuge equipped with an ultraviolet optical system with photo-electric scanner. Concentrations of DNA used for sedimentation were within the range of 20 to 30 μg/ml. The sedimentation coefficients have been corrected in the usual way to obtain $S_{20,w}$ values.

(c) *Equilibrium sedimentation in cesium chloride*

Isopycnic centrifugation was performed in CsCl solution using double-stranded T4 DNA as a density marker. To 0·5 to 1·0 μg DNA in 0·01 M-sodium citrate, solid CsCl was added until a density of 1·71 was reached. The material was sedimented for 20 hr at room temperature, at 44,770 rev./min.

(d) *Zone sedimentation in a sucrose gradient*

Sedimentation in a sucrose gradient was performed according to the procedure of Britten & Roberts (1960) in a Spinco model L preparative ultracentrifuge at 4 to 7°C with an SW39 or SW25 rotor. After centrifugation, the tubes were punctured and fractions were collected and assayed for radioactivity.

(e) *ϕX DNA spheroplasts assay*

The biological activity of purified ϕX DNA was determined according to the procedure of Guthrie & Sinsheimer (1960). Spheroplasts were prepared from *E. coli* K12 cells.

Although the specific biological activity differs from day to day, the biological activity of single-stranded and denatured double-stranded ϕX DNA is generally many times higher than that of double-stranded ϕX DNA (Jansz *et al.* 1968).

3. Results

The denaturation of double-stranded ϕX DNA was followed spectrophotometrically by measuring the absorbance at 260 mμ as a function of the pH (Fig. 1). Component II, like linear DNA molecules, shows a sharp increase of the absorbance,

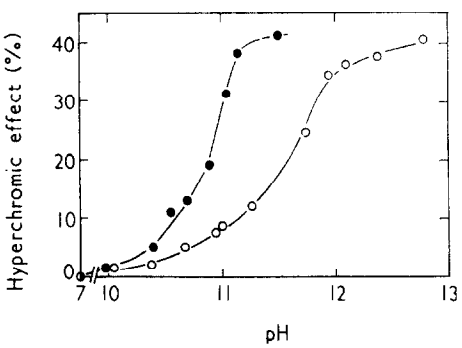

FIG. 1. Absorbance at 260 mμ *versus* pH curve for double-stranded ϕX DNA in M-NaCl–0·005 M-sodium citrate. A radiometer pH meter (electrode GK 2021, type B) was used to measure the pH. The pH values reported are uncorrected for sodium ion concentration.
(—○—○—) Component I; (—●—●—) component II.

suggesting that denaturation takes place over a small pH interval. The spectrophotometric titration curve of component I, however, is shallow, suggesting that denaturation takes place over a relatively broad pH interval. The midpoints of the increase of the hyperchromic effect differ by about 0·7 pH unit.

Sedimentation data are in fair agreement with the results from the spectrophotometric measurements. A mixture of components I and II of double-stranded ϕX DNA (ratio 3:1) was centrifuged in M-NaCl–0·005 M-sodium citrate at various pH values. The sedimentation coefficient of component I remains constant in the pH range between 7 and 11·2 (Fig. 2). Beyond pH 11·2, the sedimentation coefficient decreases and then gradually increases, reaching a plateau value (53 S) at pH 12·4. The boundaries remain sharp during centrifugation of component I as the pH is raised, suggesting that all molecules respond similarly to the change in pH. The contraction of the molecules at high pH is preceded (at pH 11·4) by an expansion of the molecules possibly because the twists, which give the molecule the unusually high sedimentation coefficient, are unwound and the molecule acquires a structure similar to component II. A similar phenomenon has been observed by Vinograd, Lebowitz, Radloff, Watson

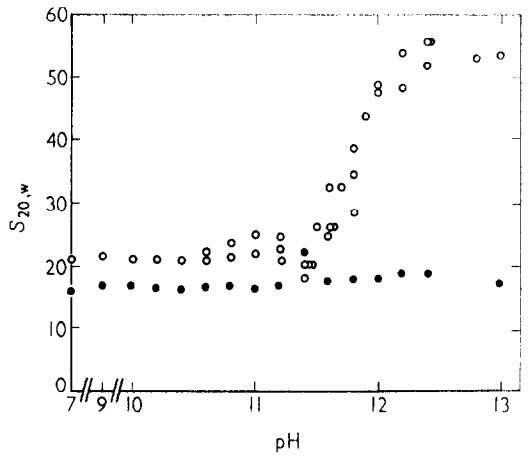

FIG. 2. Sedimentation coefficient of double-stranded ϕX DNA in M-NaCl–0·005 M-sodium citrate as a function of the solvent pH.
(○) Component I; (●) component II.

& Laipis (1965) for double-stranded DNA of polyoma virus. A similar profile of the sedimentation coefficient of component I as a function of pH, including the dip, was found in a solvent of lower ionic strength (0·1 M-phosphate buffer). The dip and the midpoint of the transition were shifted towards a higher pH by about 0·7 pH unit in this solvent as compared to M-NaCl–0·005 M-sodium citrate.

Between pH 7 and pH 11·2, the boundaries of component II also remain sharp. At higher pH the boundaries become broad, probably because the molecules are denatured at this pH. Because of the choice of sedimentation conditions, there is hardly any change in the S-value of component II upon denaturation. The midpoint of the transition from native to denatured DNA for component II is pH 11·3, in comparison to pH 11·9 for component I.

The initial increase of the sedimentation coefficient at pH 11·4 followed by a drop might be explained by a partial denaturation of the molecule. Just before strand separation occurs and the molecular weight is halved, the molecule apparently has a more contracted structure than in the native conformation (Studier, 1965).

(a) *Irreversible denaturation of double-stranded ϕX DNA*

Double-stranded ϕX DNA renatures spontaneously after heating at temperatures well above the melting temperature of component II (Pouwels & Jansz, 1964). This has been taken as evidence for the presence of two strands, which on account of their continuity cannot be separated. However, when double-stranded ϕX DNA was denatured with 0·1 N-NaOH and neutralized with acid, the material sedimented as one single component (40 S), faster than native double-stranded ϕX DNA (21 S) but significantly slower than before neutralization (53 S). Its capacity to infect spheroplasts of *E. coli* and its sensitivity to ultraviolet light have also been shown to be different from native double-stranded or single-stranded ϕX DNA (Pouwels & Jansz, 1964).

The formation and some characteristics of alkali-denatured double-stranded ϕX DNA will be described in the following paragraphs.

Double-stranded ϕX DNA labelled with ^{32}P in 0·1 M-phosphate buffer was treated with alkali and neutralized with dilute acid after 15 minutes in order to see if the two strands will recombine in proper register. By centrifugation of the material in a sucrose density-gradient in neutral M-NaCl–0·005 M-sodium citrate, it can be shown that at hydroxyl ion concentrations up to pH 12·6 component I will renature completely after neutralization of the alkali. At still higher pH values, however, component I is irreversibly denatured, giving rise to the formation of a structure sedimenting about twice as fast in this solvent as native double-stranded ϕX DNA (Fig. 3). No change in the sedimentation rate of component I was observed after denaturation at any pH between 11·4 and 12·6, even if the DNA was kept at alkaline pH for

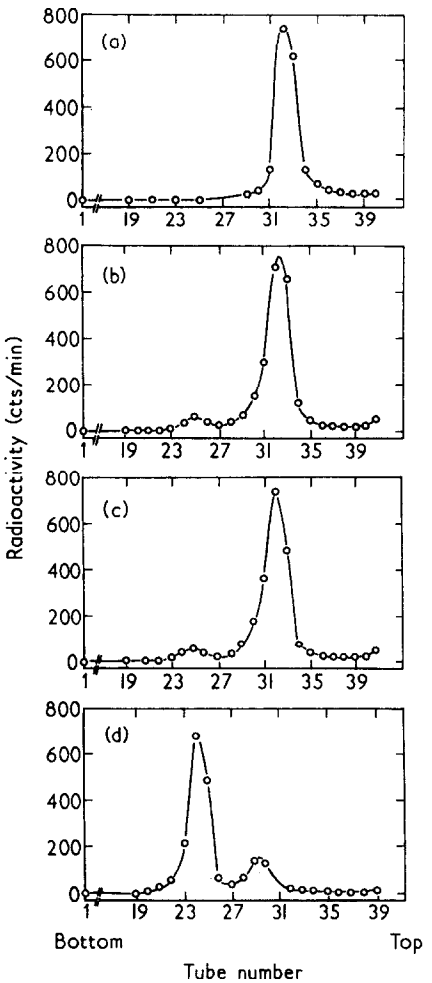

FIG. 3. Sucrose gradient centrifugation of alkali-denatured ^{32}P-labelled double-stranded ϕX DNA.

To a solution of ^{32}P-labelled double-stranded ϕX DNA (90% component I–10% component II) in 0·1 M-phosphate was added M-NaOH to pH 12·6, 12·7 and 12·8, respectively. The alkali was neutralized after 15 min by addition of M-HCl. The samples were layered on top of 29·4 ml. of sucrose solution (4 to 20% sucrose in M-NaCl–0·005 M-sodium citrate, pH 7) and spun for 6 hr at 24,000 rev./min. The tubes were punctured and fractions of 0·7 ml. were collected. The radioactivity in the various fractions was determined.

(a) DNA untreated; (b) DNA: pH 12·6 → 7; (c) DNA: pH 12·7 → 7; (d) DNA: pH 12·8 → 7.

16 hours and then neutralized. When the denaturation is performed in M-NaCl–0·005 M-sodium citrate, the irreversible denaturation of component I takes place at hydroxyl ion concentrations beyond pH 11·9 to 12·1.

The irreversible denaturation of component II was also studied using the same technique. Since single-stranded DNA can best be resolved from component I and II in buffers of low ionic strength, the samples were dialysed after denaturation and neutralization of the alkali and centrifuged in a sucrose gradient in 0·01 M-phosphate–0·001 M-sodium citrate (pH 7). In Fig. 4 is shown a sedimentation profile of a mixture

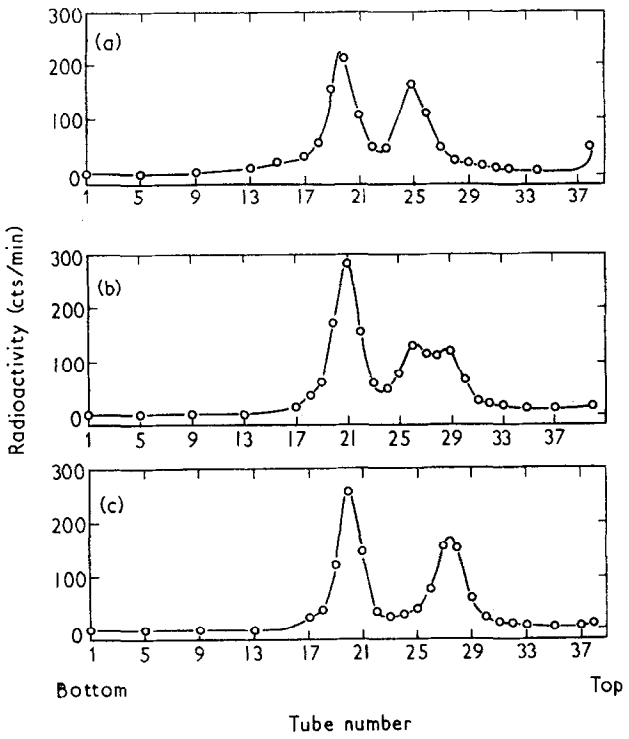

FIG. 4. Sucrose gradient centrifugation of alkali-denatured ^{32}P-labelled double-stranded ϕX DNA.

To a solution of ^{32}P-labelled double-stranded ϕX DNA (50% component I–50% component II) in M-NaCl–0·005 M-sodium citrate was added 0·1 M-NaOH to give pH 11·3 and 11·4 respectively. The alkali was neutralized after 15 min by addition of 0·1 M-HCl. The samples were dialysed against 0·01 M-phosphate–0·001 M-sodium citrate (pH 7) and layered on top of 29·4 ml. of sucrose solution (4 to 20% sucrose in 0·01 M-phosphate–0·001 M-sodium citrate, pH 7) and spun for 14 hr at 21,000 rev./min. The tubes were punctured and fractions of 0·7 ml. each were collected. The radioactivity in the various fractions was determined.

(a) DNA untreated; (b) DNA: pH 11·3 → 7; (c) DNA: pH 11·4 → 7.

of component I and II, both labelled with ^{32}P after denaturation in M-NaCl–0·005 M-sodium citrate at various pH values. It can be seen from the Figure that while the sedimentation behaviour of component I is unchanged at pH 11·4, component II is completely converted into single-stranded DNA. In 0·1 M-phosphate, component II is irreversibly denatured at pH 11·5 to 11·7†. From Figs 3 and 4 it is apparent that

† This experiment was performed using an electrode which was different from that used in all other experiments. Various species of DNA were denatured at a pH 0·1 to 0·2 unit lower than observed with the other electrode. This may explain the discrepancy that is observed between the sedimentation experiments and the biological assays (see next paragraph).

in order to denature component I irreversibly a concentration of hydroxyl ions is required which is approximately an order of magnitude higher than that required for the irreversible denaturation of component II. Since DNA from *E. coli* is denatured at a slightly higher pH ($\pm$0·1 pH unit) than component II, advantage can be taken of the high resistance of component I towards denaturation by alkali to isolate selectively native double-stranded ϕX DNA (component I) from infected bacteria in which double-stranded ϕX DNA has accumulated (Jansz *et al.*, 1966).

(b) *Biological assays*

The conversion of component I to the alkali-denatured double-stranded form and the conversion of component II to single-stranded DNA have also been studied by measuring the ability to produce phage in the spheroplast assay. The formation of denatured ϕX DNA (either double- or single-stranded) will be accompanied by an increase in the biological activity, since the specific biological activity of both single-stranded ϕX DNA and denatured double-stranded ϕX DNA is many times higher than that of native double-stranded ϕX DNA (either component I or II). In a typical experiment, a mixture of component I and component II was diluted in 0·1 M-phosphate buffer at the indicated pH and, after 15 minutes, neutralized by dilution in 0·01 M-phosphate buffer (pH 7). The biological activity was assayed using the

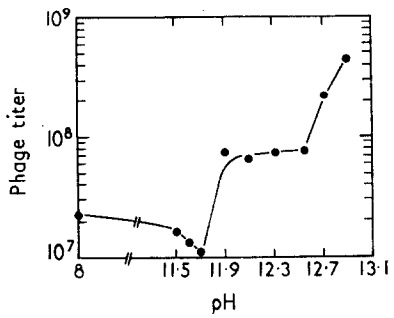

FIG. 5. Phage-forming capacity of double-stranded ϕX DNA after denaturation at various pH values.

A mixture of components I and II of double-stranded ϕX DNA was diluted tenfold in 0·1 M-phosphate buffer of increasing pH. After 15 min, the samples were neutralized by dilution in 0·01 M-phosphate (pH 7). The biological activity was determined using the spheroplast assay.

spheroplast test. The results are given in Fig. 5. It can be seen that the biological activity remains constant up to pH 11·7. The increase in infectivity at pH 11·7 to 11·9 can best be explained by the conversion of component II to single-stranded DNA. The biological activity then again remains constant up to pH 12·7. Between pH 12·7 and 12·9, the infectivity rises by a factor of five, which probably is due to the irreversible denaturation of component I to denatured double-stranded DNA. The midpoints of the transition from native double-stranded DNA to denatured single- or double-stranded DNA as measured in the biological assay are in reasonably good agreement with the midpoints of the transition determined by sucrose gradient analysis (see note on p. 174).

(c) *Properties of denatured double-stranded ϕX DNA*

Native double-stranded DNA is relatively insensitive to changes in shape when exposed to wide ranges of ionic strength, but denatured DNA is very much dependent

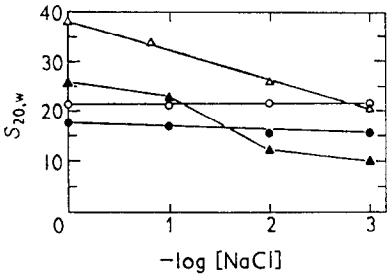

FIG. 6. Sedimentation coefficient of native and denatured double-stranded ϕX DNA as a function of the ionic strength.

(—○—○—) Native double-stranded ϕX DNA (component I); (—●—●—) native double-stranded ϕX DNA (component II); (—△—△—), alkali-denatured double-stranded ϕX DNA; (—▲—▲—) single-stranded ϕX DNA.

on the ionic environment for its hydrodynamic properties (Josse & Eigner, 1966). The presence or absence of an ordered secondary structure in DNA can be studied by a variety of techniques including velocity sedimentation, isopycnic centrifugation in CsCl, spectrophotometry and sensitivity for formaldehyde. We have used these techniques in order to explore the structure of denatured double-stranded ϕX DNA. In Fig. 6 the sedimentation coefficients of alkali-denatured double-stranded ϕX DNA are plotted as a function of the ionic strength. For comparison the sedimentation coefficients of native double-stranded ϕX DNA (component I and II) and single-stranded ϕX DNA are also given.

The strong dependence of the sedimentation coefficient of denatured double-stranded ϕX DNA on the salt concentration is typical for DNA lacking an ordered secondary structure, like single-stranded ϕX DNA, but differs significantly from the results obtained with native double-stranded ϕX DNA. Moreover, the observed sedimentation coefficient in 0·15 M-NaCl–0·015 M-sodium citrate is in good agreement with results found for other denatured DNA molecules of that size (Eigner & Doty, 1965). From Fig. 6 it can also be seen that the sedimentation coefficient of component II shows a small dependence on the ionic strength, which also has been observed for native linear DNA molecules (Studier, 1965), but the sedimentation coefficient of

TABLE 1

Buoyant density of ϕX DNA in cesium chloride

Native double-stranded ϕX DNA (component I or II)	1·706 g/cm^3
Denatured double-stranded ϕX DNA	1·718
Single-stranded ϕX DNA (phage DNA)	1·724
Single-stranded ϕX DNA (denatured component II)	1·718

component I is virtually independent of the ionic strength. It is conceivable that, because of the highly twisted structure, component I is less flexible than component II, which shows a more extended circular structure.

After centrifugation to equilibrium in a gradient set up in CsCl, a single symmetrical peak was observed at a buoyant density of 1·718 g/ml. (Table 1). The density found for denatured double-stranded DNA equals the buoyant density of single-stranded

ϕX DNA liberated from component II by denaturation with alkali or by heat treatment, but differs significantly from the density of native double-stranded ϕX DNA or of single-stranded DNA isolated from mature phage.

When DNA having an ordered secondary structure is exposed to elevated temperatures, denaturation takes place in a co-operative manner. This can be demonstrated by an abrupt increase of the absorbance at 260 mμ as the temperature is raised over a small temperature range. DNA lacking an ordered secondary structure shows a

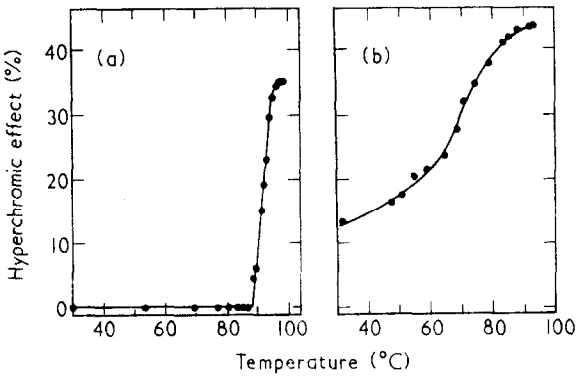

FIG. 7. Temperature–absorbance curve of native and denatured double-stranded ϕX DNA in 0·15 M-NaCl–0·015 M-sodium citrate (pH 7).
(a) Native double-stranded ϕX DNA; (b) alkali-denatured double-stranded ϕX DNA.

gradual increase of the absorbance at much lower temperature. In Fig. 7 profiles are given of the absorbance at 260 mμ as a function of the temperature for native double-stranded ϕX DNA (component II) and alkali-denatured double-stranded ϕX DNA. It can be seen from this Figure that native double-stranded DNA melts over a small temperature range, but the melting profile of denatured double-stranded DNA is typical for denatured or single-stranded DNA.

(d) *Reaction with formaldehyde*

Formaldehyde has been shown to be a useful tool in exploring the secondary structure of nucleic acids, since it will react with the amino-groups of the bases A, G and C, when DNA is in the denatured conformation. In native DNA, the amino-groups are protected against formaldehyde by hydrogen bonds. The reaction product of the bases with formaldehyde shows an absorbance maximum at 275 mμ (Grossman, Levine & Allison, 1961). In Fig. 8 is plotted the absorbance at 275 mμ as a function of the time of incubation at 30°C of denatured double-stranded ϕX DNA in 0·1 M-phosphate buffer (pH 6·7) with formaldehyde (1% solution). For comparison, the data for single-stranded ϕX DNA liberated from component II by heat denaturation are also given. It can be seen from Fig. 8 that denatured double-stranded ϕX DNA reacts to an appreciable extent with formaldehyde, although the reaction is much slower as compared to single-stranded DNA.

(e) *Sensitivity of denatured double-stranded ϕX DNA to pancreatic DNase*

Denatured double-stranded DNA will renature spontaneously even at low temperature in solvents of low ionic strength, after introduction of single-strand breaks, as will be demonstrated by the following experiments.

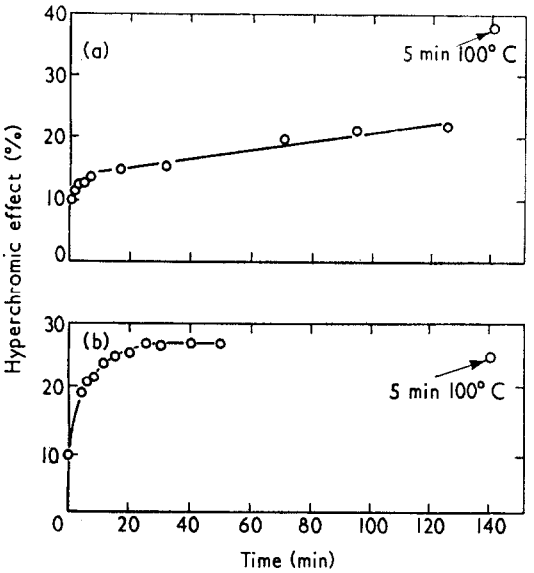

FIG. 8. Reaction of ϕX DNA with formaldehyde.

Double-stranded ϕX DNA (component I and II) was denatured with alkali and, after dialysis against 0·1 M-phosphate buffer (pH 6·7), incubated at 30°C with a 1% solution of formaldehyde. Absorbance at 275 mμ was read at various times of incubation and is given as percentage hyperchromic effect. In the Figure is also given the percentage hyperchromic effect which is observed after heating of the samples at 100°C for 5 min.

(a) Denatured double-stranded ϕX DNA (component I); (b) denatured double-stranded ϕX DNA (component II).

When a mixture of component I and II, labelled with ^{32}P, is denatured with alkali at pH 12·8 and after neutralization of the alkali is treated with small amounts of pancreatic DNase, denatured double-stranded DNA is converted into a structure which sediments significantly more slowly (Fig. 9). After denaturation with alkali, the slow-sedimenting material is converted into a structure which now sediments more rapidly than before denaturation.

The following arguments favour the hypothesis that the product of limited DNase digestion of denatured double-stranded DNA is native double-stranded DNA (component II).

(i) After conversion with DNase, the material sediments in M-NaCl with $S=16{\cdot}8$ s, a value which is close to that found for component II (17·2 s). The sedimentation coefficient is almost constant over a wide range of the ionic strength.

(ii) The material is capable of producing phage when incubated with spheroplasts of *E. coli*. The specific biological activity is similar to that of native double-stranded ϕX DNA.

(iii) After heat denaturation, the capacity to produce phages in the spheroplast assay is increased (about fivefold in the experiment cited in Fig. 9).

(iv) The sensitivity of the material to ultraviolet light is equal to that of native double-stranded ϕX DNA.

(v) After denaturation with alkali, the material sediments at the position of single-stranded ϕX DNA: the sensitivity for ultraviolet light of this material resembles

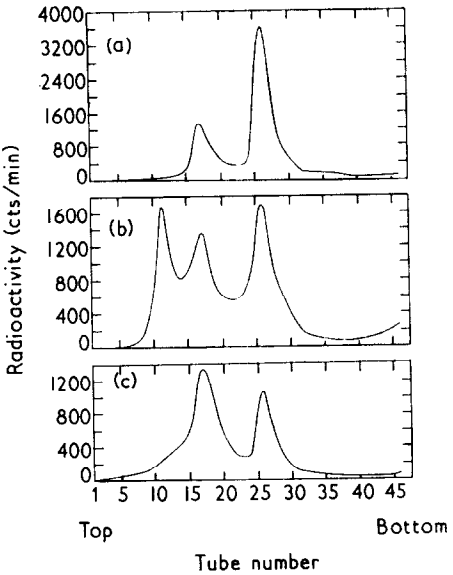

FIG. 9. Sucrose gradient centrifugation of alkali-denatured ^{32}P-labelled double-stranded ϕX DNA after DNase treatment and denaturation with alkali.

A mixture of ^{32}P-labelled component I and II of double-stranded ϕX DNA was denatured with alkali and incubated with 0·004 μg DNase/μg DNA/ml. After incubation, part of the material was denatured with 0·1 N-NaOH. The materials were layered on top of 4·8 ml. of sucrose solution (5 to 23% sucrose in M-NaCl–0·005 M-sodium citrate, pH 7) and spun for 2·5 hr at 39,000 rev./min. The tubes were punctured and fractions of 0·1 ml. were collected. The radioactivity in the various fractions was determined.

(a) Double-stranded ϕX DNA denatured with alkali; (b) *idem*, after incubation with pancreatic DNase; (c) as (b), followed by denaturation with alkali.

closely that of single-stranded DNA liberated from component II after heat- or alkali-denaturation.

(vi) The material is denatured over a small temperature range with a T_m of 69·6°C in 0·01 M-phosphate–0·001 M-sodium citrate (pH 7).

(vii) In a density gradient set up in CsCl, one peak of material is observed at a buoyant density of 1·707 g/cm^3.

(viii) Electron micrographs of preparations of denatured double-stranded ϕX DNA treated with DNase show the presence of open circles and linear molecules (probably component III) (Jansz *et al.*, 1968).

4. Discussion

Previous studies on the effect of pancreatic DNase on double-stranded ϕX DNA have led us to propose that the two forms of phage DNA found in infected cells are both circular molecules in which no single-strand scissions (component I) or one or more single-strand scissions (component II) are present. This model has been further substantiated by the present studies on the alkali denaturation of components I and II.

Component II is irreversibly denatured in M-NaCl if the pH is raised to pH 11·3 or higher, giving rise to biologically active single-stranded DNA, as could be demonstrated by a combination of sedimentation analysis and biological assays. The conversion of native double-stranded DNA to single-stranded DNA takes place over a sharp pH interval (probably within 0·1 pH unit) in contrast to the denaturation of component I. Component I in M-NaCl is denatured over a wide pH range (1·0 pH

unit) and is irreversibly denatured at pH 11·9 to 12·1 or higher, which is 0·6 to 0·8 pH unit higher than required for the denaturation of component II in this solvent. In a buffer of lower ionic strength, the denaturation of both component I and II takes place at a higher pH. Dependence of the transition from native to denatured DNA on the ionic strength has already been observed by Shack & Thompsett (1952) and Cox & Peacocke (1956), and has been attributed to a binding of counter ions to specific sites of the polynucleotide. The displacement of the denaturation curves with increasing ionic strength was explained by assuming that cations replace the hydrogen ions that are released. From velocity sedimentation experiments, Vinograd *et al.* (1965) and Vinograd & Lebowitz (1966) concluded that in circular double-stranded polyoma DNA (component I), left-handed turns are superimposed on the right-handed duplex turns. Molecules which contain left-handed superhelical turns are converted spontaneously into right-handed twisted structures. A similar situation seems to hold for double-stranded ϕX DNA. In the early stages of denaturation, some of the duplex turns unwind. The unwinding of the Watson–Crick double helix is accompanied by an unwinding of the twists. This will first lead to a structure in which no twists are present and therefore will be indistinguishable from component II with respect to sedimentation behaviour. As denaturation proceeds, left-handed twists are forced into the molecule, giving it a more compact structure. This is seen as a decrease of the sedimentation coefficient at pH 11·4 followed by a gradual increase.

In velocity sedimentation experiments, the boundaries of partly or completely denatured double-stranded ϕX DNA remain sharp, indicating that the material is homogeneous with respect to conformation. This, as well as the fact that the reversibility of denaturation of double-stranded DNA is lost at a well-defined pH, suggests that the irreversible denaturation of component I is a process analogous to that of denaturation of linear DNA molecules, with the exception that the strands do not separate. The high resistance of component I towards alkali denaturation points to the fact that interconversion of two conformations of DNA, i.e. native and denatured, is hindered as long as both polynucleotide strands are continuous. In preliminary experiments, it was found that renaturation of denatured double-stranded DNA does not occur under conditions where linear DNA's from phage easily renature. On the other hand, denatured double-stranded ϕX DNA renatures spontaneously, once a single-strand break is introduced, as is revealed by the DNase experiments (see below).

The sudden loss of reversibility at a well-defined pH value may be explained by a shift of the two strands with respect to each other along the longitudinal axis of the helix, after the hydrogen bonds of the last nucleotide pair have been broken. When the alkali is neutralized, non-specific base pairing will occur. The molecule will therefore be locked, since a shift back to the original position, which is required for proper renaturation, is prevented by non-specific base pairs.

Another possible explanation, however, is that because of the high number of tertiary turns which are present after denaturation with alkali, the molecule is sterically hindered from folding back into a helical conformation.

At neutral pH, denatured double-stranded ϕX DNA shows features which are characteristic of denatured DNA. The sedimentation coefficient of denatured double-stranded ϕX DNA is strongly dependent on the ionic strength, as is the sedimentation coefficient of single-stranded ϕX DNA. Native double-stranded ϕX DNA shows a

very small dependence (component II) and virtually no dependence (component I) of the sedimentation coefficient on the ionic strength. Also the gradual increase of the absorbance as a function of the temperature and the high buoyant density in CsCl suggest the absence of an ordered secondary structure in denatured double-stranded ϕX DNA. A small but consistent difference in buoyant density was found between single-stranded DNA from mature phage and single-stranded DNA liberated from double-stranded DNA by denaturation. The reason for this difference is as yet obscure and is at present being investigated. A decrease of the sedimentation coefficient of denatured double-stranded DNA (53 S $\rightarrow$ 40 S) after neutralization of the alkali may therefore be explained by a reduction of the number of tertiary turns rather than by partial re-formation of a double-helical structure.

A remarkable feature of denatured double-stranded ϕX DNA is its ability to renature spontaneously after introduction of a single-strand break, by means of pancreatic DNase. From the melting behaviour, the buoyant density in CsCl, the sedimentation coefficient in M-NaCl, the infectivity in the spheroplast assay, the sensitivity to ultraviolet light before and after heating, and finally the electron micrographs, it is concluded that the product of limited digestion by pancreatic DNase of denatured double-stranded ϕX DNA is component II of double-stranded ϕX DNA. In preliminary experiments it was found that the conversion of denatured double-stranded ϕX DNA to component II could also be accomplished when single-strand scissions were introduced by means of X-rays, heat or acid pH. Under certain conditions (various nucleolytic enzymes and reducing agents such as cysteine) other products were obtained besides component II, which are at present being investigated. From the kinetics of the reaction, Jansz *et al.* (1968) tentatively conclude that one single-strand break is sufficient to facilitate the renaturation of denatured double-stranded ϕX DNA to component II. The rapid renaturation of denatured double-stranded DNA may be explained by assuming that the introduction of a single-strand break generates a swivel which allows the unwinding of the tertiary twists. Because of the close proximity of the two complementary strands, specific base pairing is facilitated and renaturation may proceed. Once a nucleus of paired bases is formed, renaturation will proceed at low temperature. An analogous situation exists when the two strands of linear DNA have been united by a cross-link before denaturation (Geiduschek, 1961). This structure has been called reversible DNA.

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