STRUCTURE OF THE REPLICATIVE FORM OF BACTERIOPHAGE ØX174

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Recent studies on the double-stranded replicative form of ØX174 DNA (1) indicate that it occurs in a ring structure (2,3,4) with separately continuous strands (5).

Purified preparations of double-stranded ØX DNA show a high degree of homogeneity upon ultracentrifugal analysis; at least 90% sediments as a single component with an $S_{20,w}$ value of 21 S (4,5). The value of 21 S is rather high for rod-shaped DNA molecules of the molecular weight (3.4×10^6) of double-stranded ØX DNA (3,4), but seems to be a reasonable estimate for cyclic DNA molecules of this size (6,7,8).

Sedimentation analysis of our ØX DNA preparations (5) in 1 M NaCl-0.01 M phosphate buffer pH 7.0 using the band-centrifugation technique of Vinograd et al. (9) showed the presence of a second component ($S_{20,w}$ of 16.0 S $\pm$ 0.4) besides the major component with $S_{20,w}$ of 20.7 S $\pm$ 0.5. The second component represented in different preparations 0-20% of the total material. Similar results were obtained by boundary-sedimentation analysis. The two components will be designated as component I (21 S) and component II (16 S) respectively.

The biological and physico-chemical features of component I and II which will be presented in this paper indicate that component II is derived from component I by random and single breaks in either one of

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the two strands of the cyclic helix i.e. the first degradation product is composed of one broken and one continuous strand.

The two components can be separated by centrifugation in a sucrose-gradient in 0.01 M phosphate buffer pH 7.0 (Fig. 1). The biological

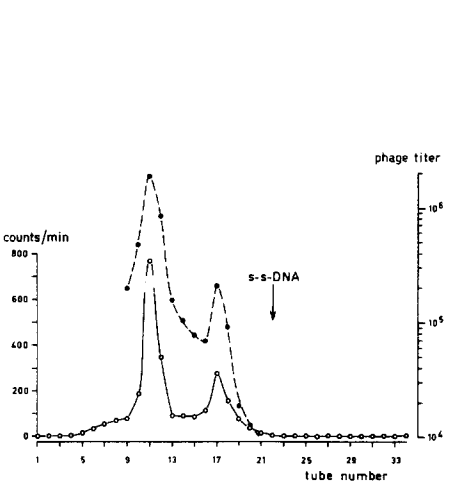

Fig. 1

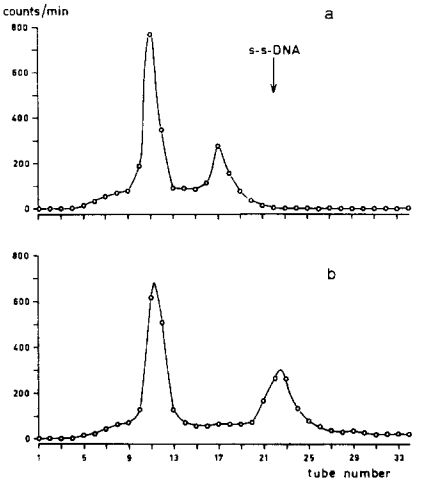

Fig. 2

Fig. 1. Sedimentation pattern of double-stranded ØX DNA. Double-stranded ØX DNA (^{32}P-labeled) was isolated from infected cells with phenol and purified by means of methylated albumin-celite columns. A sample of this material was layered on top of 25 ml sucrose solution (4-20% in 0.01 M phosphate buffer pH 7.0) and spun for 14 hours at 21.000 rev/min. The tube was punctured and fractions of 0.7 ml were collected. The radioactivity and biological activity in the various fractions were determined. Solid line: radioactivity (counts/min); broken line: biological activity (phages/ml). The arrow shows the position of single-stranded ØX DNA (s-s-DNA).

Fig. 2. Sucrose-gradient centrifugation of native double-stranded (a) and heat denatured double-stranded ^{32}P-ØX DNA. For details see Fig. 1.

assay (10) of the various fractions obtained after centrifugation indicates that component I as well as component II is infectious for spheroplasts of Escherichia coli. The biological activity per weight unit of DNA of the two components is of the same order of magnitude. It is known (5) that single-stranded DNA is 10-100 times more active in the spheroplast assay than double-stranded DNA.

The high resistance of component II to inactivation by ultraviolet light is equal to that of component I which characterizes component II as double-stranded DNA (1,11,12).

When double-stranded ØX DNA is heated for 5 minutes at 90° C in 0.01 M phosphate pH 7.0 and rapidly cooled it was found that component II had been converted into single strands whereas component I is not affected as can be seen from the experiment presented in Fig. 2. This is in agreement with an earlier finding (5) that only a small fraction of double-stranded ØX DNA can be converted to single strands under conditions of denaturation. The single-stranded material thus originates from component II.

By treatment of double-stranded ØX DNA with a small amount of DNase, component I is converted into component II. This could be shown by sucrose-gradient centrifugation of samples of double-stranded (^{32}P-labeled) ØX DNA that had been treated with DNase for various lengths of time (Fig. 3). It will be observed that the material (^{32}P label) of component I decreases and that of component II increases with time of DNase digestion. Also the biological activity of component I decreases whereas that of component II increases, indicating that small amounts of DNase do not impair the overall biological activity. The conversion of component I to component II is a logarithmic function of time of DNase digestion as can be seen from the insert to Fig. 3.

DNase treated double-stranded ØX DNA (^{32}P-labeled) was heated for 5 minutes at 90° C in 0.01 M phosphate - 0.001 M citrate pH 7.0 and subjected to centrifugation in a sucrose-gradient in the same buffer. The result of this experiment is presented in Fig. 4. Obviously component II has been converted completely to single-stranded DNA as indicated by the fact that this material sediments at the position of single-stranded DNA. It is significant that the single-stranded DNA thus obtained is biologically active. Since Fiers and Sinsheimer (13) have shown that circularity of single-stranded DNA is a prerequisite for biological activity it seems likely to conclude that component II contains circular molecules.

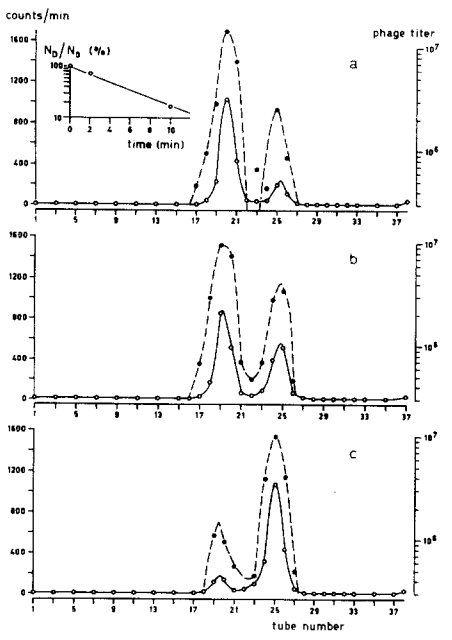

Fig. 3

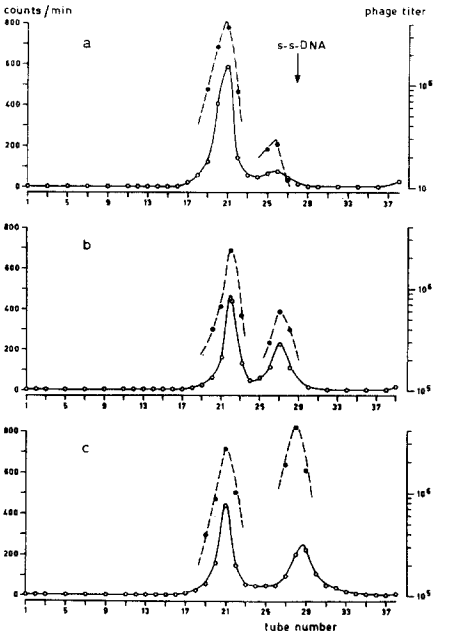

Fig. 4

Fig. 3. The effect of DNase on the sedimentation pattern of double-stranded ØX DNA. ^{32}P-labeled double-stranded ØX DNA (15 µg/ml) was incubated at 25° C with 0.0002 µg DNase/ml in 0.01 M phosphate pH 7.0 - 0.001 M $MgCl_2$ for 0, 2 and 10 min. The reaction was terminated by adding Na-citrate to a final concentration of 0.01 M. The samples were layered on top of 29.4 ml of sucrose solution (4-20% in 0.01 M phosphate buffer pH 7.0 - 0.001 M Na-citrate) and spun for 14 hours at 21.000 rev/min. Further conditions as described in Fig. 1. Solid line: radioactivity (counts/min); broken line: biological activity (phages/ml). a) 0 min DNase b) 2 min DNase c) 10 min DNase. In the insert the fraction of component I that has survived DNase action (N_D/N_0) is plotted against time of DNase action.

Fig. 4. The sedimentation pattern of double-stranded ØX DNA after DNase treatment and heat denaturation. Double-stranded ØX DNA (^{32}P-labeled; 15 µg/ml) was incubated at 25° C with 0.0002 µg DNase/ml for 2 min and the reaction was terminated by adding Na-citrate. Part of the sample was heated for 5 min at 90° C and quickly cooled. Further conditions as described in Fig. 3. a) double-stranded ØX DNA, b) idem, 2 min DNase, c) idem, 2 min DNase, 5 min 90° C. The arrow shows the position of single-stranded ØX DNA (s-s-DNA).

All these observations are in agreement with the following model: Component II is derived from component I by random and single breaks in either one of the two continuous strands of component I i.e. the first degradation product is composed of a double helix with one open and one continuous strand. Why a single chain scission leads to such a profound effect on the S-value of a cyclic molecule is not known.

The results obtained from the kinetics of DNase digestion give further support to the model proposed. In Fig. 5 the lower curve re-

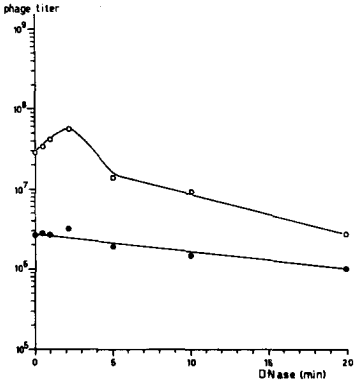

Fig. 5

Fig. 5. Inactivation of double-stranded ØX DNA by DNase. The survival of the biological activity of double-stranded ØX DNA has been plotted as a function of the time of DNase action. Double-stranded ØX DNA (15 µg/ml) was incubated with 0.0002 µg DNase/ml for various lengths of time and the biological activity was assayed (lower curve). Part of the samples was heated (5 min 90° C) after DNase digestion before the assay of the biological activity (upper curve).

presents the relation between the activity in the spheroplast assay of double-stranded ØX DNA and the time of DNase action. The upper curve (Fig. 5) is a plot of the activity of DNase treated double-stranded ØX DNA that had been heated before the biological assay. It will be observed that at the early times of DNase treatment the activity of the unheated samples is relatively unaffected, whereas heating induces a profound change in biological activity. The biological activity initially increases up to a maximum value and then decreases. This is in agreement with the model proposed. The initial chain breaks which convert component I to component II will increase the amount of single-stranded material that can be obtained upon heat denaturation, thus promoting an increase in biological activity. From Fig. 5 it will be observed that the slope of the curve of the heated sample is steeper than that of the unheated material. The difference in slope has been found to be significant in other experiments. This may indicate that chain breaks in double-

stranded ØX DNA do not influence the activity but become lethal after heat denaturation.

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