COHESION OCCURRING BETWEEN DNA MOLECULES OF TEMPERATE PHAGES φ80 AND LAMBDA OR φ81 *

Hideo Yamagishi**, Keiko Nakamura and Haruo Ozeki

Department of Chemistry, National Institute of Health of Japan, Shinagawa-ku, Tokyo, Japan

Received August 16, 1965

Molecules of phage lambda DNA form rings and chains by their terminal cohesive sites, which are probably single-stranded polynucleotide extensions whose base sequences are complementary between two ends of each molecule (Hershey, Burgi and Ingraham, 1963; Hershey, 1964; Hershey and Burgi, 1965). In this report, we examine whether the terminal cohesion takes place or not between DNA molecules of different phages, namely φ80, φ81 and lambda, after confirming the ring formation of φ80DNA and φ81DNA.

Temperate phages φ80 and φ81 were isolated from E. coli strain B80(see Matsushiro, 1963), and λc was supplied by the courtesy of Dr.E.Kellenberger. The hybrid phage($i^{80}h^{\lambda}$) used, carrying the immunity determinant of φ80 and the host range determinant of λ, was isolated in our laboratory(see Signer, 1964). Our genetic data suggested that the hybrid phage was probably derived from an exchange of arms between φ80 and λ(Ozeki and Nakamura, unpublished; see Franklin, Dove and Yanafsky, 1965).

Bacteriophage DNA was prepared from purified phage particles by treatment with phenol(Frankel, 1963) followed by dialysis against standard saline citrate(1xSSC). Half-length fragments of DNA molecules were obtained by stirring an ice-cold solution of 5μg/ml of linear monomer DNA in 1xSSC

---

* Supported partly by a research grant GM 08384 from the United States Public Health Service.

** On leave of absence from the Department of Applied Biology, Radiation Center of Osaka Prefecture, Sakai, Osaka, Japan.

at 6,000 r.p.m. for 45 minutes with a laboratory stirrer. To bring about joining of DNA molecules at their cohesive ends, the DNA solution in 1xSSC or in 0.6M NaCl-0.05M phosphate buffer(pH 6.7) was incubated at 75°C for 10 minutes and allowed to cool slowly to 30°C in the heating bath with the heater disconnected. In order to disjoin the cohered ends, the DNA solution was quickly cooled in an ice-bath after the heating at 75°C for 10 minutes; this was done shortly before use. Relative molecular weights of phage DNAs were determined by sucrose gradient centrifugation at 14°C using λDNA as a reference(Burgi and Hershey, 1963); the values obtained are,

$\lambda : \phi 80 : \phi 81 : i^{80}h^{\lambda} = 1.0 : 0.95 : 1.0 : 1.05.$

Figure 1 shows the sedimentation patterns of quickly cooled(Fig. 1A) and slowly cooled ^{32}P-φ80DNA(Fig. 1B), after thermal treatment at a concentration of 0.5μg/ml in 1xSSC, mixed with quickly cooled ^{3}H-φ80DNA as a reference marker. In the slowly cooled sample, a narrow band moving 1.13 times faster than the quickly cooled linear molecules was observed. Similar patterns were also obtained with φ81 and $i^{80}h^{\lambda}$ DNA. In comparison

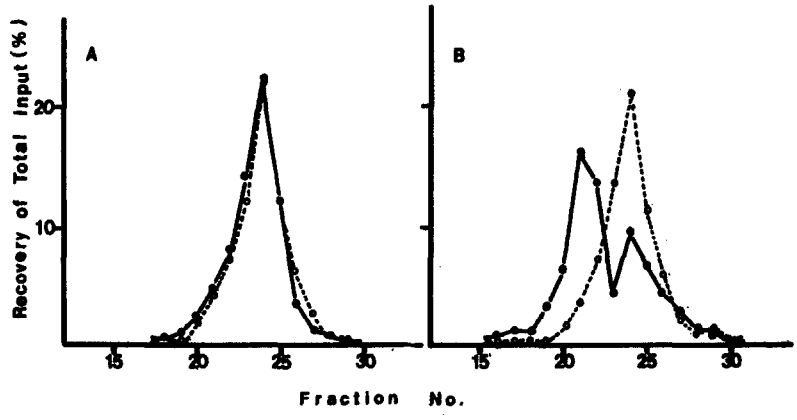

Fig. 1. Zone sedimentation of φ80DNA. Broken lines: ^{3}H-φ80 linear monomer DNA as a reference. Solid lines: ^{32}P-φ80DNA heated at 75°C for 10 minutes and quickly cooled(A), and ^{32}P-φ80DNA heated at 75°C for 10 minutes and slowly cooled(B). Thermal treatment was done at a concentration of 0.5μg/ml of DNA in 1xSSC. 0.1 ml of the DNA sample was layered on 4.8 ml of sucrose solution and spun for 5 hours at 30,000 r.p.m. in an SW39 rotor with Spinco Model L ultracentrifuge at 14°C.

with the data on λDNA(Hershey et al., 1963; MacHattie and Thomas, 1964), this band could consist of circular monomers. Later, this was confirmed by electronmicroscopy with φ80DNA(Hosaka, Shinagawa and Yamagishi, to be published) and with φ81DNA(Tomizawa and Hara, personal communication).

In experiments similar to those in Figure 1B, but where the concentration and salinity of DNA solution had been raised to 10μg/ml and 0.6M NaCl, respectively, bands sedimenting 1.25-1.30 and 1.43-1.50 times faster than the linear molecules appeared in addition to the circular monomer band. These sedimentation rates correspond to the values expected for dimers and trimers, respectively(Hershey et al., 1963). These polymer formations also occurred between DNAs of different phages, such as between a tracer amount of ^{3}H-λDNA and a carrier amount of unlabeled φ80 or φ81 DNA, but not with T2 DNA. These results suggest the homologous nature of the cohesive sites of λ-, φ80- and φ81-DNA. This point was confirmed in the following experiments, in which the joining of sheared half-molecules of one phage with either half- or complete-molecules of another phage was attempted.

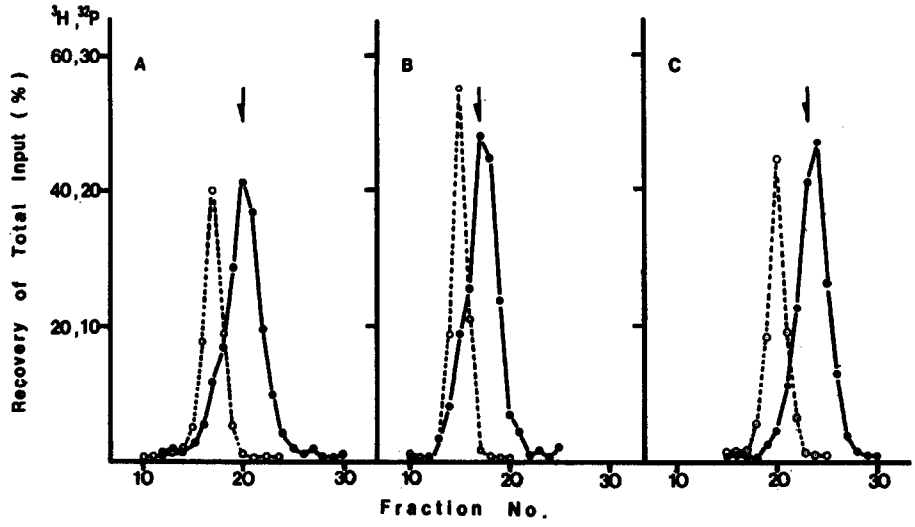

Fig. 2. Zone sedimentation of sheared fragments of φ80DNA. Broken lines: ^{3}H-φ80 linear monomer DNA as a reference. Solid lines: sheared fragments of the mixture of ^{32}P-φ80DNA and 5 times the amount of unlabeled DNA of φ80(A), λ(B) or φ81(C). Each sample was stirred at the concentration of 5μg/ml in 1xSSC. 0.2 ml of the sample was layered on 4.8 ml of sucrose solution and spun for 4.5 hours at 30,000 r.p.m. at 14°C. Arrow shows the position of half-length fragments.

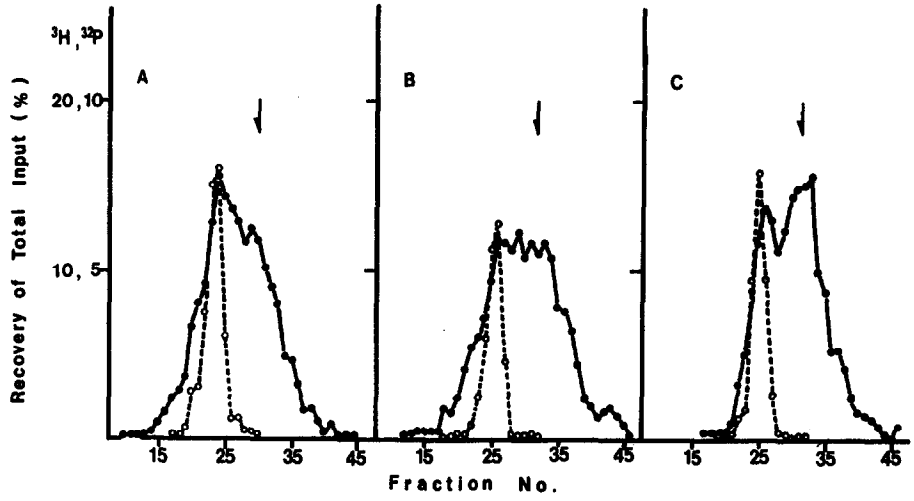

Fig. 3. Zone sedimentation of sheared ^{32}P-ϕ80 DNA after thermal treatment with 5 times the amount of unlabeled fragments of ϕ80(A), λc(B), or ϕ81(C) to join molecular ends(solid lines). ^3H-λc linear monomer DNA as a reference(broken lines). Each sample was slowly cooled after incubation at 75°C for 10 minutes at the concentration of 5μg/ml in 0.6M NaCl-0.05M phosphate (pH 6.7). 0.1 ml of the sample was layered on 4.8 ml of sucrose solution and spun as in Fig.2. Arrow shows the original position of half-length fragments.

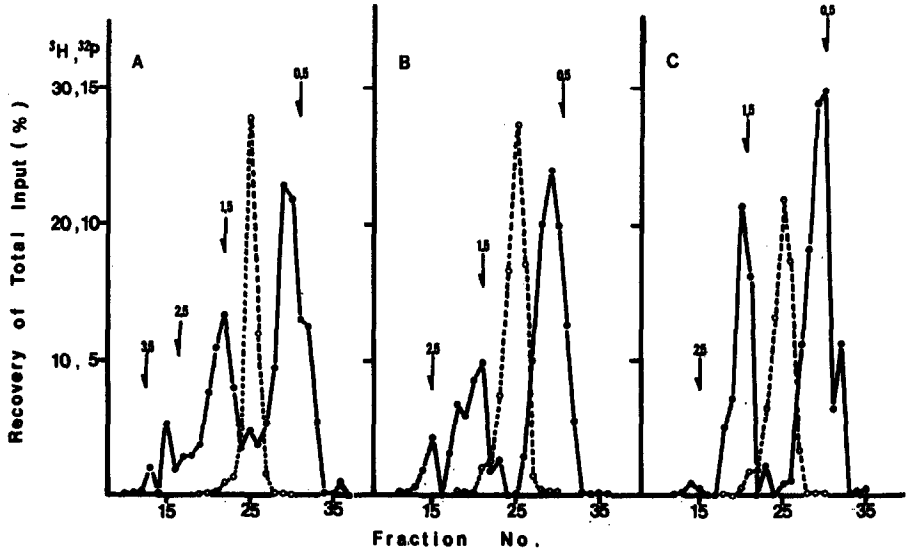

Fig. 4. Zone sedimentation of ^{32}P-ϕ80 DNA fragments after thermal treatment with added carrier of complete linear monomers of ϕ80(A), λc(B), or ϕ81(C) to join molecular ends(solid lines). As a reference, ^3H-ϕ80 linear monomer DNA was used(broken lines). Thermal treatment and centrifugation were carried out as in Fig. 3. Arrows show the positions of 0.5, 1.5, 2.5 and 3.5 lengths molecules.

The mixture of ^{32}P-ϕ80DNA and 5 times the amount of unlabeled DNA of either ϕ80, λc or ϕ81 was sheared to half-length fragments and subjected to sucrose gradient centrifugation(Fig. 2). Each of these samples was slowly cooled after heat treatment and examined for its sedimentation pattern(Fig.3). In all patterns, a considerable amount of ^{32}P-ϕ80DNA was found at the position of the linear monomer of ϕ80DNA, while the rest of the labeled DNA remained at the original position. No component sedimenting much faster than the reference peak was observed.

The half-length fragments of ^{32}P-ϕ80DNA were mixed with unlabeled complete DNA molecules of either ϕ80, λc, or ϕ81 in the same concentration as in the previous experiments and the sedimentation patterns were examined after joining those molecules(Fig. 4). A considerable fraction of ^{32}P-DNA formed new broad bands which sedimented 1.16-1.20 and 1.40-1.45 times faster than the reference marker of linear monomer of ^{3}H-ϕ80DNA. The former sedimentation rate is in agreement with that expected from the joining of a half-fragment and a complete linear monomer, i.e. 1.5 lengths of DNA, and the latter corresponds to 2.5 lengths of linear DNA. In these two experiments, the changes in sedimentation rate of ^{32}P-DNA(half fragments) were dependent upon the length of unlabeled DNA molecules added in excess, and not upon the kind of phage employed. Thus it is concluded that DNAs of different temperate phages are able to cohere mutually by their ends.

The formation of circular molecules of $i^{80}h^{\lambda}$ DNA also supports the above conclusion, since the DNA molecule seems to have two ends, one derived from ϕ80 and the other from λphage, as is suggested from genetic data. It might be further speculated from this observation that the right cohesive ends of ϕ80 is complementary with the left cohesive ends of λ, and vice versa, in their parallel genetic maps in respect to the h and i markers.

Three kinds of joined molecules of half-fragments obtained in the above experiments, namely $\frac{1}{2}\phi$80-$\frac{1}{2}\phi$80, $\frac{1}{2}\phi$80-$\frac{1}{2}\lambda$ and $\frac{1}{2}\phi$80-$\frac{1}{2}\phi$81, were completely disjoined to half-length molecules by stirring at 3,000 r.p.m., while 6,000

r.p.m. was required to shear the complete molecules to halves at a 5μg/ml concentration of DNA. Thus the difference in stability to shearing force between homologous and heterologous joined molecules could not be detected in this type of experiment, although it revealed the fragile nature of the jointed points.

In summary, it was shown that the DNA molecules of φ80 and φ81 have cohesive ends which are comparable to that of λ, and moreover terminal cohesion takes place between these different phage DNAs.

Acknowledgements. The authors would like to thank Dr. J. Tomizawa for his continuous interest and pertinent criticisms during the course of this work.

REFERENCES

Burgi, E. and Hershey, A.D., Biophysical J. 3, 309 (1963)
Frankel, F. R., Proc. Natl. Acad. Sci. 49, 366 (1963)
Hershey, A. D., Carnegie Inst. Wash. Year Book 63, 580 (1964)
Hershey, A.D., Burgi, E. and Ingraham, L., Proc. Natl. Acad. Sci. 49, 748(1963)
Hershey, A. D. and Burgi, E., Proc. Natl. Acad. Sci. 53, 325 (1965)
MacHattie, L. A. and Thomas, C. A., Science 144, 1142 (1964)
Matsushiro, A., Virology 19, 475 (1963)
Signer, E. R., Virology 22, 650 (1964)