On the Sequence Similarity of the Cohesive Ends of Coliphage P4, P2, and 186 Deoxyribonucleic Acid†

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ABSTRACT: The thermal stability of the end-to-end aggregates between the satellite phage P4 DNA and P2 DNA, and between P4 DNA and 186 DNA, was studied. The results indicate that the base sequences of the cohesive ends of P4 DNA and P2 DNA are identical. The base sequences of the cohesive ends of P2 DNA and 186 DNA are similar but not identical. A thermodynamic calculation shows that the cohered ends of P2 DNA have at least one more G·C pair than the cohered ends of 186 DNA. The thermal stability of P4–186 end-to-end aggregates is consistent with the notion that the cohered ends of P4 and 186 may differ by as little as a single G·C → A·T change.

Satellite bacteriophage P4 requires all the known head and tail genes of a helper phage such as P2 for synthesis of its own head and tail (Six, 1963; Six and Lindqvist, 1970; Six, 1973[1]). Temperate coliphages P2, 186, 299, PK, and Wϕ all serve as helpers for P4 (Six and Klug, 1973; Six, 1972[2]). As far as is known, all these helper phages contain double-stranded DNA with closely related 5′-phosphoryl-terminated complementary ends (Mandel and Berg, 1968a,b; Padmanabhan and Wu, 1972[3]). P4 DNA itself also possesses cohesive ends (Inman *et al.*, 1971).

It is generally believed that the cohesive ends are generated by an enzyme system which makes two staggered single-chain cuts at unique sites. The separation of the hydrogen-bonded base pairs between the two single-chain scissions gives two single-stranded complementary ends. For λ DNA, it is believed that the gene A product is involved in the generation of the cohesive ends (Wang and Kaiser, 1973). Since mutants defective in gene A are blocked in phage head synthesis (Weigle, 1966; Mackinlay and Kaiser, 1969; Murialdo and Siminovich, 1972), it is likely that the synthesis of phage head and the generation of cohesive ends are intimately related. Since P4 requires all the late genes of a helper phage, its cohesive ends are probably generated by the same enzyme system responsible for the generation of the cohesive ends of the helper phage itself. Therefore, P4 DNA should carry cohesive ends similar to those of the helper phage DNAs.

Two types of experiments can be performed to test the sequence similarity of the cohesive ends of two DNAs. Base sequence analysis of the ends (Wu and Taylor, 1971; Padmanabhan and Wu, 1972) provides direct information, although the procedures are tedious and time consuming. As an alternative, the formation of mixed dimers or higher aggregates when two DNAs are annealed is indicative of close similarity in sequences (Yamagishi *et al.*, 1965; Baldwin *et al.*, 1966; Wang and Schwartz, 1967; Mandel and Berg, 1968a,b). The latter type of experiments is easy to perform but usually does not give precise sequence information. Nevertheless, considerable sequence information can be extracted from this type of experiment when properly performed. In this article, we report results on the sequence similarity of the cohesive ends of P4, P2, and 186 DNA obtained by examining the formation and the thermal stability of mixed dimers and higher aggregates between the DNAs.

Experimental Procedure

Materials. Phage 186 was obtained by thermal induction of W3550 (186 p), originally obtained from Dr. R. L. Baldwin. ^{3}H-labeled P2 *vir*$_1$ phage were obtained by lytic infection as described by Lengyel *et al.* (1973). Each phage sample was purified by two cycles of differential centrifugation and then banded in CsCl for 24 hr. The phage band collected was dialyzed against 0.01 M Tris (pH 7.4)–0.01 M $MgCl_2$, and extracted twice with an equal volume of phenol saturated with 0.1 M Tris, pH 8, each time. The DNA samples were dialyzed exhaustively against 0.1 M NaCl–0.01 M Na_3EDTA. Unlabeled P2 *vir*$_1$ DNA was kindly given to us by Dr. J. Geisselsoder.

Zone Sedimentation. Zone sedimentation was done on linear 5–20% sucrose gradients containing 0.01 M Tris (pH 8)–0.01 M NaCl. Usually 0.1 ml of a DNA solution was layered on each gradient and centrifugation was done at 4° and 40,000 rpm (SW 50.1 rotor) in a Model L2 65B ultracentrifuge (Spinco). The rotor was coasted down after 4 hr and fractions were collected and counted in a scintillation counter as described previously (Saucier and Wang, 1972). The relatively low temperature and ionic strength of the sedimentation medium were chosen so that no appreciable joining of the cohesive ends would occur during sedimentation.

Other Methods. Shear breakage of P2 DNA into halves was done as described previously (Wang and Schwartz, 1967). Sonication of the DNA solution (0.5 ml) was performed using a sonicator (Heat Systems-ultrasonics, Inc., Model W185) with a needle probe. The DNA solution was kept in an ice bath during two 30-sec sonic bursts and the power output of the sonicator was set close to the minimal setting.

Results

To test whether a P4 DNA molecule can join to a P2 or 186 DNA molecule, ^{3}H-labeled P4 and unlabeled P2 or 186 DNA were annealed and the distribution of the ^{3}H label was ex-

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[1] Six, E. W. (1973), manuscript in preparation.

[2] Six, E. W. (1972), personal communication.

[3] Padmanabham, R., and Wu, R. (1972), personal communication.

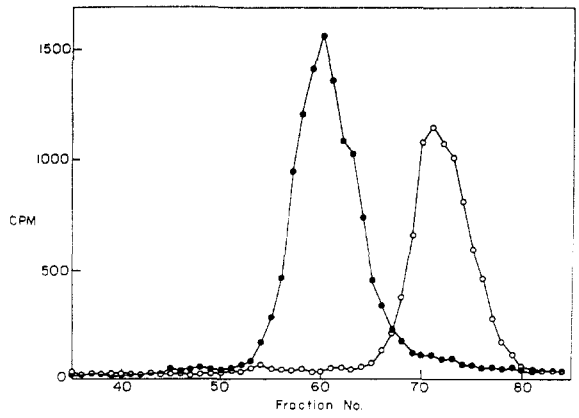

FIGURE 1: Sucrose gradient sedimentation pattern of a mixture of linear ^{32}P-labeled P2 DNA (●) and ^{3}H-labeled P4 DNA (○). The direction of sedimentation in this and all other figures is from right to left.

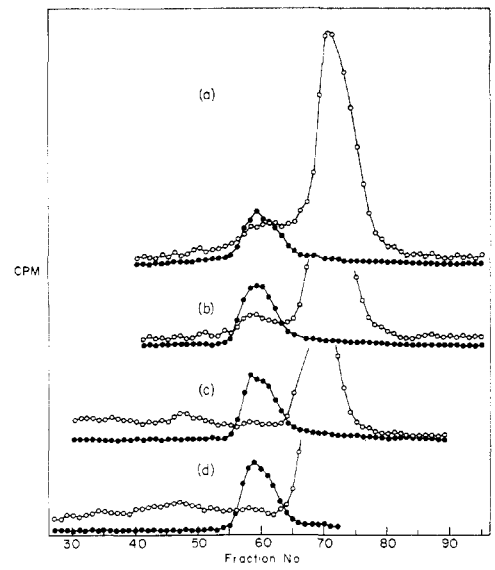

FIGURE 2: Sucrose gradient sedimentation patterns of ^{3}H-labeled P4 DNA annealed (a) by itself, (b) with unlabeled λb2b5c DNA, (c) with unlabeled P2 DNA, and (d) with unlabeled 186 DNA. In each case 50 μl of a solution containing 1.9 μg/ml of P4 DNA and 19 μg/ml of the other DNA in 0.1 M NaCl–0.01 M Na_3EDTA, pH 8, was first disaggregated by heating at 75° for 5 min and cooled quickly to 0°, and then annealed at 45° for 4 hr. The annealed mixture was then mixed with an equal volume of ^{32}P-labeled P2 DNA which had been disaggregated to the linear monomer form by heating and quick cooling: ○, ^{3}H activity; ●, ^{32}P activity.

amined after sedimenting the mixture on a 5–20% sucrose gradient. The molecular weight of P4 DNA is approximately one-third of that of P2 DNA (22×10^6, Inman and Bertani, 1969) or 186 DNA (19.7×10^6, Wang, 1967). Inman *et al.* (1971) gave the molecular weight of P4 DNA as 6.7×10^6. Our electron microscopy measurements, with λb2b5c as an internal marker, gave a value of $(7.47 \pm 0.2) \times 10^6$, with the molecular weight of λb2b5c taken as 25.8×10^6 (Davidson and Szybalski, 1971). The end-to-end joining of P4 to P2 or 186 DNA should therefore give faster sedimenting ^{3}H-labeled species. The shorter length of P4 DNA, however, is associated with a higher probability of cyclization, which is inversely proportional to the $^{3}/_{2}$ power of the molecular length (Wang and Davidson, 1966a,b, 1968). Therefore, in order to have a significant amount of P4 DNA joined to P2 or 186 DNA, the concentration of the latter should be considerably higher. In all annealing experiments, the concentration of P2 or 186 DNA by mass was usually tenfold higher than P4 DNA.

Figure 1 depicts the sedimentation pattern of a mixture of ^{3}H-labeled P4 DNA and ^{32}P-labeled P2 DNA disaggregated by heating at 75° for 5 min (in 0.1 M NaCl–0.01 M Na_3EDTA) followed by quickly cooling to 0°. The relative sedimentation rates are calculated to be 1.00 and 1.41, respectively, for P4 and P2 DNA. In subsequent calculations, the relative sedimentation rate of the linear P2 DNA is taken as 1.41.

Figures 2a–d depict the zone sedimentation patterns of ^{3}H-labeled P4 DNA annealed by itself and with unlabeled λb2b5c, P2, and 186 DNA, respectively. The ^{32}P activity is linear ^{32}P-labeled P2 DNA added after the annealing to serve as a sedimentation marker. As shown in Figure 2a, when P4 DNA is annealed by itself, the bulk of the radioactivity sediments as a sharp peak with a relative sedimentation rate of 1.09. Comparing with a relative sedimentation rate of 1.00 for linear P4 DNA, the sedimentation rate of the major species indicates that it is monomeric circular P4 DNA. There is a minor band sedimenting at the same rate as linear P2 DNA, or 1.28 times that of the major P4 peak. This ratio indicates that the minor peak is dimeric circular P4 DNA. (For a review on the sedimentation properties of linear and circular DNA, *cf.* Bloomfield, 1968.)

The sedimentation pattern of ^{3}H-labeled P4 DNA annealed with λb2b5c DNA (Figure 2b) is very similar to that of ^{3}H-labeled P4 annealed by itself (Figure 2a). Two conclusions can be drawn. Firstly, the cohesive ends of P4 DNA do not join with the cohesive ends of λb2b5c DNA. Secondly, from the known thermodynamics and kinetics of the cyclization of λb2b5c DNA, the annealing at 45° in the salt medium results in the cyclization of λb2b5c DNA (Wang and Davidson, 1966a). Since both P4 and λb2b5c DNA are cyclized, a comparison between Figure 2a and 2b shows that the amount of interlocked rings between the two DNAs is small. This is expected from the estimated equilibrium constant of catenane formation (Wang and Schwartz, 1967).

The sedimentation pattern of ^{3}H-labeled P4 DNA annealed with P2 DNA (Figure 2c) or 186 DNA (Figure 2d) is quite different from the previous cases (Figure 2a and 2b). A significant amount of counts sediments faster than linear P2 DNA. Since the amount of interlocked rings is small, the species which sediments faster than linear P2 DNA must result from the joining of the P4 DNA to P2 or 186 DNA *via the cohesive ends*. It can therefore be concluded that the base sequences of the cohesive ends of P4, P2, and 186 DNA are very similar, while the cohesive ends of λb2b5c DNA are of a different class. It is known that the sequences of the cohesive ends of λb2b5c DNA and 186 DNA are different from direct sequence results (Wu and Taylor, 1971; Padmanabhan and Wu, 1972), the thermodynamics and kinetics of cyclization (Wang and Davidson, 1966a; Wang 1967; Wang and Schwartz, 1967), and the helper phage assay results (Kaiser and Wu, 1968; Mandel and Berg, 1968a,b). The helper assay and mixed aggregates formation results on P2 and 186 further suggest that the cohesive ends of these two DNAs are similar. The current results are in agreement with these previous observations.

The demonstration of joining of two DNAs with cohesive ends (under conditions such that the formation of interlocked rings is insignificant) indicates that the sequences of the ends are similar or identical. More information can be obtained, however, by examining the thermal stability of the joined form. Figures 3a–d depict the sedimentation patterns of ^{3}H-

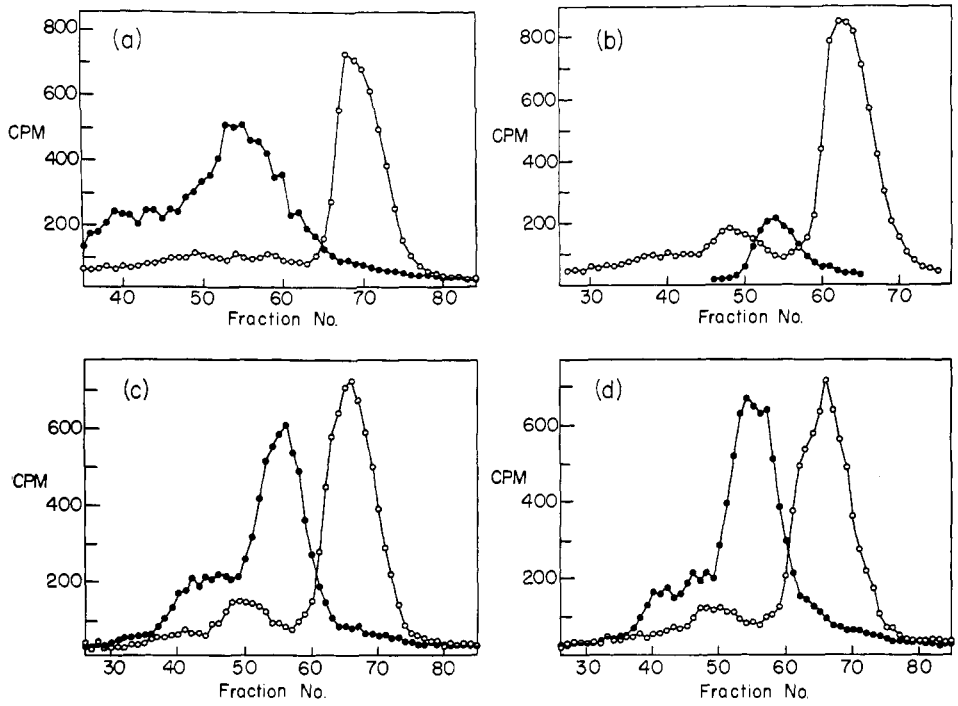

FIGURE 3: Zone sedimentation patterns of ^{3}H-labeled P4 DNA annealed with P2 DNA at (a) 55.5°, (b) 60°, (c) 63°, and (d) 66° in 0.1 M NaCl–0.01 M Na_3EDTA, pH 8. In b, the P2 DNA in the annealing mixture was unlabeled and ^{32}P-labeled linear P2 DNA was added afterward as a marker. In all other cases the P2 DNA was ^{32}P labeled. The annealing time was 90 min at 55.5° and 20 min at the higher temperatures: O, ^{3}H activity; ●, ^{32}P activity. Background counts are ~20 cpm in ^{32}P and 30 cpm in ^{3}H and they have not been subtracted from the total counts. Approximately 100 fractions were collected for each gradient.

labeled P4 DNA annealed with P2 DNA at 55.5, 60, 63, and 66° in 0.1 M NaCl–0.01 M Na_3EDTA. The melting temperature T_m for the conversion of circular P2 DNA to the linear form in this medium is estimated to be 65.5° from published results (Mandel and Berg, 1968b; Wang and Davidson, 1968). Oligomeric circles are less stable since there are n ways to linearize a cyclic n-mer, and since the unfavorable entropy of cyclization increases with increasing chain length (Jacobson and Stockmayer, 1950).

It can be seen from Figure 3 that the thermal stability of aggregates between P4 and P2 DNA is *qualitatively* comparable to the cohered ends of P2 DNA. At 66°, the estimated T_m of circular P2, a significant fraction of P4 remains joined to at least one P2 DNA and therefore sediments faster than a linear P2 DNA. The implication from these results is that the cohesive ends of P4 and P2 DNA are identical (see Discussion).

While a quantitative treatment of the thermal stability of the various species is possible in principle from theory of ring–chain equilibrium (Jacobson and Stockmayer, 1950; Wang and Davidson, 1966a,b), the presence of a multitude of species makes such an analysis difficult. In order to reduce the number of possible species, we annealed P2 DNA half-molecules with small fragments of labeled P2 and P4 DNA. Unlabeled P2 DNA was sheared to halves hydrodynamically. Calculated amounts of ^{32}P-labeled P2 DNA and ^{3}H-labeled P4 DNA were mixed to give approximately equal numbers of *molecules* of each DNA and the mixture was sonicated to give DNA fragments with an average molecular weight of ~10^6. The sonicated mixture was then annealed with unlabeled P2 DNA halves such that the labeled cohesive ends are in excess compared with the unlabeled cohesive ends of the P2 halves. If the ends of P2 and P4 are identical, equal amounts of ^{32}P- and ^{3}H-labeled ends would join to the P2 DNA halves. The sedimentation result for a sample annealed at 60° is shown in Figure 4. Approximately equal amounts of the ^{3}H- and ^{32}P-labeled ends are found to join to the halves. This result is again in agreement with the notion that the cohesive ends of P4 and P2 DNA are identical.

Figures 5a–d depict the sedimentation patterns of ^{3}H-labeled P4 DNA annealed with 186 DNA at 50, 55.5, 60, and 63° in 0.1 M NaCl–0.01 M Na_3EDTA. A comparison between Figures 3 and 5 clearly indicates that P4 DNA joined to 186 DNA is less stable than P4 DNA joined to P2 DNA. The thermal stability of the former is approximately 10° less than that of the latter. Therefore, the cohesive ends of 186 DNA and P4 DNA are similar but not identical.

Discussion

Mandel and Berg (1968b) have shown that the melting temperature of the cohered ends of P2 DNA, T_m^{P2}, is 71° in 0.195 M Na^+, or 2.5° above that of the cohered ends of 186 DNA, T_m^{P186}. A thermodynamic analysis of ring formation (eq 5 in Wang, 1967) gives

$$n^{P2}(T_m'^{P2} - T_m^{P2}) = \frac{1}{\Delta s}\{(T_m^{P2}\,\Delta S_c^{P2} - T_m^{186}\,\Delta S_c^{186}) + n^{186}(T_m'^{186} - T_m^{186})\Delta s\} \quad (1)$$

where n, T_m', and ΔS_c are, respectively, the number of bases per cohesive end, the melting temperature of a large DNA of the same base composition as the cohered ends, and the entropy of cyclization. The superscripts P2 and 186 designate quantities for P2 and 186, respectively. The quantity Δs is the entropy change per mole of base pair for the formation of a double-stranded DNA helix from the complementary single strands. The molecular weights of 186 DNA and P2 DNA are

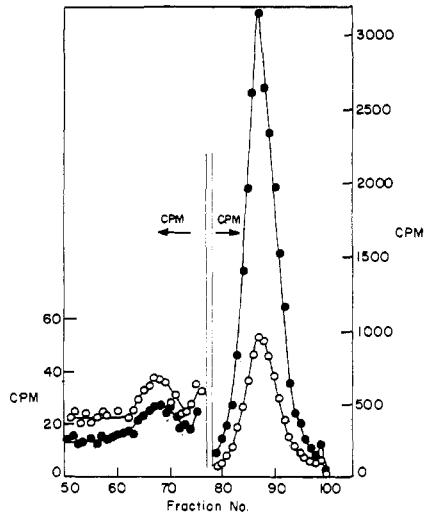

FIGURE 4: Sedimentation pattern of a mixture of sonicated ^{3}H-labeled P4 DNA, ^{32}P-labeled P2 DNA, and unlabeled P2 DNA halves after annealing at 60° for 30 min in 0.1 M NaCl–0.01 M Na_3EDTA. The mixture was disaggregated prior to annealing by heating at 70° for 5 min. The DNA concentrations in the mixture were 1.45, 4.35, and 5.35 μg/ml, respectively, for sonicated P4 DNA, sonicated P2 DNA, and P2 DNA halves. In a similar experiment, the annealing was done at 50° for 90 min. An increase in both ^{3}H and ^{32}P counts in the faster sedimenting band was observed. As a control, the P2 DNA halves were first annealed at 50° for 90 min to promote the joining of the halves. The solution was then mixed with sonicated ^{32}P-labeled P2 DNA fragments (which had been disaggregated), annealed at 50° for another 90 min, and sedimented. The amount of ^{32}P label in the faster sedimenting band was greatly reduced. This shows that the fragments are joined to the DNA halves mainly *via* the cohesive ends.

similar. Therefore, the entropy of cyclization, which is proportional to the logarithms of the $^{3}/_{2}$ power of the molecular weight (Wang and Davidson, 1966a,b), is approximately the same for the two DNAs. Since T_m^{P2} is also close to T_m^{186}, the term $(T_m^{P2}\Delta S_c^{P2} - T_m^{186}\Delta S_c^{186})$ can be neglected. This gives

$$n^{P2}(T_m'^{P2} - T_m^{P2}) = n^{186}(T_m'^{186} - T_m^{186}) \qquad (2)$$

It is known that there are 13 G·C pairs and 6 A·T pairs in the cohered ends of 186 DNA. $T_m'^{186}$ is therefore calculated to be 373°K from the dependence of the melting temperature on the base composition (Marmur and Doty, 1959). For a given n^{P2}, $T_m'^{P2}$ and therefore the per cent G·C of the cohesive ends can be calculated. The total number of G·C pairs per cohered P2 ends, $n_{G\cdot C}^{P2}$, is the product of n^{P2} and the per cent G·C.

Equation 2 requires that n^{P2} be at least 14, corresponding to 100% G·C for the ends. Furthermore, $n_{G\cdot C}^{P2}$ calculated from eq 2 is nearly independent of n^{P2}. In the range n^{P2} = 14–26, for example, the calculated $n_{G\cdot C}^{P2}$ ranges from 14.2 to 14.1. This result is not surprising, since the melting temperature of a large DNA of pure A·T is very close to T_m^{P2}. Therefore, the addition of A·T pairs has little effect on the stability of the cohered P2 ends. The conclusion is that there are at least 14 G·C pairs per cohered ends of P2 DNA. Due to the errors in the measured T_m values and the approximations made in arriving at eq 2, the possibility that there are 15 G·C pairs per cohered ends of P2 DNA cannot be ruled out, but more than 15 G·C pairs are highly unlikely.

We shall now consider the effect of a mismatch in otherwise perfectly joined 186 ends. The reduction of the total number of base pairs from 19 to 18 *per se* reduces the T_m but little, especially if the total number of G·C pairs remains to be 13. A mismatched pair, however, causes an interruption in the originally perfect helix and a reduction of the stacking interaction is expected. This causes a rather large drop in the thermal stability. Precise datum is lacking on the free energy of a stacking interaction but various estimates indicate that the range is between −3 and −7 kcal (Wang and Davidson,

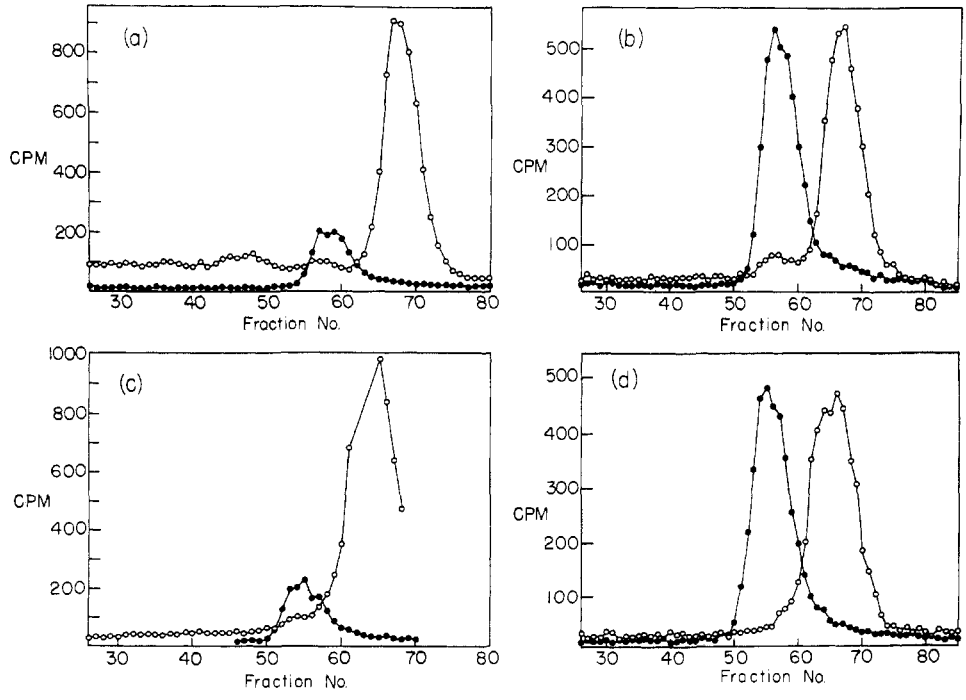

FIGURE 5: Zone sedimentation patterns of ^{3}H-labeled P4 DNA annealed with unlabeled 186 DNA at (a) 50°, (b) 55°, (c) 60°, and (d) 63°. The medium and the annealing conditions have been described in the legend to Figure 3. ^{32}P-Labeled linear P2 DNA was added to each sample after annealing to serve as a marker: ○, ^{3}H activity; ●, ^{32}P activity.

1966a,b 1968; Davidson and Szybalski, 1971). The loss of a stacking interaction would reduce T_m^{186} by ~7 to 17° (*cf.* Wang and Davidson, 1968). Uhlenbeck *et al.* (1971) have shown that for the self-complementary oligoribonucleotides $(Ap)_7C(pU)_7$ and $(Ap)_6ApU(pU)_6$, the melting temperature of the double-stranded dimer of $(Ap)_7C(pU)_7$ is lower by ~10° compared with that of $(Ap)_6ApU(pU)_6$, even though the total number of base pairs is the same. We have shown that the thermal stability of P4 DNA joined to 186 DNA is ~10° lower than that of P4 DNA joined to P2 DNA. This indicates that there is probably only one interruption in the cohered ends between a P4 DNA and a 186 DNA. The above discussion indicates that the sequences of the cohesive ends of P2 (and therefore P4) and 186 are very similar. The cohered ends of 186 and P2 DNA may differ by as little as the replacement of an A·T pair in the former by a G·C pair in the latter. If there are more than one mismatched pair in a 186–P2 join, they are likely clustered in one region.

Since a single mismatched pair is expected to cause a rather large drop in T_m, the thermal stability of P4 joined to P2 strongly suggests that the sequences of the cohesive ends of the DNAs are identical.

As pointed out in the introductory statement, the cohesive ends of P4 are likely to be generated by the same enzyme system responsible for the generation of the cohesive ends of the helper phage, such as 186. The similar but not identical sequences of the ends of P4 and 186 therefore raise an interesting point with regard to the recognition of the cutting sites by the enzyme system. If the sequences of the ends of the two DNAs differ in a region important in the recognition process, the efficiency of the enzyme system would be reduced. We note that 186 is a poor helper for P4 relative to P2 (Six, 1972[2]), but whether this is a direct consequence of the difference in the sequences of the ends is uncertain.

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