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Studies on nucleic acid reassociation kinetics: Reactivity of single-stranded tails in DNA-DNA renaturation

(S1 nuclease/hydroxyapatite)

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ABSTRACT **The reassociation kinetics of *Escherichia coli* DNA were measured by S1 nuclease resistance and hydroxyapatite binding. While the reaction assayed by hydroxyapatite displays second order kinetics, the S1 nuclease measurements follow a non-second order form, as previously reported by Morrow (Ph.D. Dissertation, Stanford University, 1974). Much of the reaction measured with S1 nuclease occurs between single stranded regions of fragments already bearing duplex structures from previous collisions, and between such regions and totally free single strands. Experimental determinations indicate that the nucleation rate of single stranded regions on fragments also containing duplexes is inhibited by an average factor of 2 to 4.**

The methods most commonly used for the measurement of eukaryotic DNA reassociation kinetics are binding to hydroxyapatite (HAP), measurement of hypochromicity, and assay of resistance to single strand specific nucleases. Generally the S1 nuclease of *Aspergillus oryzae* is used (1, 2), though other single strand specific nucleases such as Mung bean nuclease (3) have also been applied. The nuclease procedures measure the fraction of DNA nucleotides (NT) in duplex at each point in the renaturation reaction. At least to a first approximation this should also be true of the optical hypochromicity method. On the other hand, HAP chromatography measures the fraction of DNA fragments that are totally single stranded. For randomly sheared DNA, the structures bound to HAP must contain both single stranded and duplex regions, since in general no two reacting fragments begin and end at the same point in the DNA sequence.

With the increasing use of S1 nuclease for the assay of duplex formation, somewhat better accuracy is possible, and it has become useful to consider the actual kinetics of duplex formation. Morrow (4) reported that the kinetics of reassociation of randomly sheared simian virus 40 DNA fragments assayed with S1 nuclease are significantly different from second order. After the early part of the reaction, a slower rate of formation of duplex is observed than would be predicted from the initial rate if second order kinetics were followed. In the experiments described here we have directly compared the kinetics of *Escherichia coli* DNA reassociation as measured by HAP and by S1 nuclease resistance. Our results for the appearance of S1 nuclease resistance show quantitatively the same kinetic form as do Morrow's measurements. In order to understand the causes of the observed kinetics, a number of further studies have been undertaken. In particular we have explored the rates of reaction of the single strand ends of partially reassociated structures. These are termed "particles" since late in the reaction they can grow to fairly large size (Graham, Eden, Davidson, and Britten, in preparation). Reactions of such unpaired regions with each other and with single strands on totally unpaired fragments (termed "free") form an important part of the reassociation process which has not been fully analyzed in previous treatments. The experiments presented in this paper show that the nucleation rate for these unpaired regions is retarded by a factor of 2 to 4, compared to the rate of reaction of totally single stranded fragments.

Abbreviations: PB, phosphate buffer; HAP, hydroxyapatite; NT, nucleotides; C_0t, concentration of DNA (moles NT per liter) × sec.

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MATERIALS AND METHODS

Extraction and Purification of *E. coli* DNA. DNA was extracted from *E. coli* cell paste (Miles Laboratories) or from commercial *E. coli* DNA preparations (Worthington Biochemicals) by standard methods. All preparations were treated with Pronase B (CalBiochem) and RNase A (Worthington). [^{3}H]DNA (specific activity 1.5×10^5 cpm/μg) was extracted from *E. coli* grown for 4 cell generations (2.5 hr) in glucose minimal salts solution (5) and incubated with [^{3}H]thymidine (10 μCi/ml).

DNA Shearing and Size Determination. *E. coli* [^{3}H]DNA was diluted 30-fold with unlabeled DNA prior to shearing. The mixture was made 0.15 M in Na acetate at pH 6.5 and sheared twice at 12,500 psi (86.3 MPa) in the Britten high pressure press. The sheared [^{3}H]DNA was passed over a Chelex 100 column and precipitated with ethanol from 0.3 M Na acetate (pH 6.5). [^{3}H]DNA was loaded on preparative isokinetic alkaline sucrose gradients and centrifuged at 41,000 rpm for 24 hr at 10°. Parallel gradients contained DNA markers of known size previously determined by electron microscopy (6, 7). [^{3}H]DNA peaks of average size 750 NT were pooled and dialyzed overnight against 0.3 M Na acetate at pH 6.5. The DNA was precipitated with ethanol and the pellet taken up in 0.12 M phosphate buffer (PB).

The unlabeled DNA and [^{3}H]DNA utilized in the experiments described in Table 1 were sheared in a Virtis model 60 homogenizer under conditions that give DNA lengths of 1000 and 300 NT, respectively (8). Fragment sizes of the final preparations were measured on alkaline sucrose gradients.

DNA Reassociation and HAP Chromatography. *E. coli* [^{3}H]DNA in PB was denatured in sealed vials and immediately transferred to a 60° water bath for renaturation. Routinely the PB concentration was maintained at 0.12 M except in cases of extremely high C_0t (DNA concentration in moles of NT liter^{-1} times sec). In these cases the PB concentration was increased up to 0.5 M. The C_0t corrected for acceleration of renaturation rate due to higher cation concentration (8) is the equivalent C_0t of the reaction. At the selected C_0t, the [^{3}H]DNA samples were removed from the 60°

Table 1. Experimental observation of rate inhibition in reaction of particle single strands compared to free single strands

1	2	3	4	5	6	7	8	9
		Calculated length of single stranded regions on driver particles at C_0t[a]	Fraction of driver DNA at C_0t					
Driver DNA equivalent C_0t when tracer DNA added (M sec)	Starting driver fragment length L_D(NT)	L_R(NT)	HAP unbound	S1 nuclease sensitive[b]	Self-reassociation rate of driver DNA[c] K_D (M^{-1} sec^{-1})	Expected rate of tracer association with driver[d] K_T(exp) (M^{-1} sec^{-1})	Observed rate of tracer association with driver[h] K_T(obs) (M^{-1} sec^{-1})	Minimum inhibition factor K_T(exp)/K_T(obs)
20	1000	250	0.055	0.277	0.516	0.0791[e]	0.044	1.8
1000		100	0.025	0.067		0.0328[f]	0.013	2.5
20 (HAP bound DNA used as driver)	600	160	0.02	(0.39)	0.393	0.148[g]	0.064	2.3

300 NT long tracer DNA is added to driver DNA at indicated driver DNA C_0ts, and rate of association with driver DNA is measured by HAP chromatography.

[a] Average length of single strand regions on particles (L_R) was calculated by an empirical relationship described in the second paper of this series†.

[b] S1 sensitivity was measured directly except for value in parentheses, which was calculated from Eq. 2 using the measured driver rate (0.393 liter $moles^{-1}$ sec^{-1}). For the other cases the measured values compare closely to the expected values calculated from Eq. 2. Thus expected values for the driver reacting at the rate of 0.516 liter $moles^{-1}$ sec^{-1} are 0.34 for C_0t 20 and 0.076 for C_0t 1000.

[c] Driver reassociation rates (K_D) were obtained as usual from HAP measurements. The values given are the best least squares fits to Eq. 1.

[d] Calculations of the expected rates of tracer-driver association [$K_{T(exp)}$] on HAP utilize a result obtained by Galau, Klein, Smith, Britten and Davidson (in preparation). There it is shown that when driver DNA is longer than tracer the rate of reaction of a tracer of length L_T with a driver of length L_D is given approximately by $K_D(L_T/L_D)$, where K_D is the driver rate constant. For the C_0t 0 tracer additions, K_D is the measured driver rate (column 6). Where the tracer is added at C_0t 20 or C_0t 1000, K_D must be calculated from the lengths shown in column 3 since the length of the particle single strands, L_R, is less than the starting length, L_0. Here the usual $L^{1/2}$ relationship must be applied. Thus for these cases

$$K_D = K_D \text{ (column 6)} \left\{ \frac{300}{[L_R \text{ (column 3)}]} \right\}^{1/2}$$

(tracer length L_T is 300 NT throughout). Individual calculations are given in the following notes.

[e] To calculate the expected tracer rate we must know that fraction of the total single stranded DNA driver (0.277, column 5) which is particle single strand and that fraction which is free single strand, since the rates of these two components of the overall tracer reaction will be different. The reason for this is that the average lengths of the free single strand driver is different from that of the particle single strand driver. We calculate† the average free single strand length is 440 nucleotides at C_0t 20, while that of the particle single strand is 250 nucleotides. Eq. 1 tells us that 0.088 of the DNA fragments renaturing at a rate of 0.516 M^{-1} sec^{-1} is present as free single strands at C_0t 20 (this is a more reliable value for the calculation than the terminal estimate seen in column 4, 0.055). Thus about 0.189 of the DNA is particle single strand (0.277 − 0.088 = 0.189). For each fraction of the tracer reaction we calculate the expected driver rate K_D as in note [d] above: for the free single strands $K_D = 0.088 \times 0.51\ M^{-1}\ sec^{-1} \times (440/1000)^{1/2} = 0.0301\ M^{-1}\ sec^{-1}$; for the particle single strands $K_D = 0.189 \times 0.516\ M^{-1}\ sec^{-1} \times (250/1000)^{1/2} = 0.0488$. $K_{T(exp)}$ for the free single strands is then $0.0301\ M^{-1}\ sec^{-1}\ (300/440) = 0.0205\ M^{-1}\ sec^{-1}$ and $K_{T(exp)}$ for the particle single strands is $0.0488\ M^{-1}\ sec^{-1}\ (300/250) = 0.0586$. The overall value of $K_{T(exp)}$ is the sum of these two reactions or $0.079\ M^{-1}\ sec^{-1}$.

[f] The fraction of free single strands at C_0t 1000 is so small that only the particle single strand reaction need be considered. The calculation is otherwise the same as in note [e]. Thus $K_{T(exp)} = 0.516\ M^{-1}\ sec^{-1}$ (column 6) × 0.067 (column 5) × [100 (column 3)]/1000 (column 2)$^{1/2}$ × [300/100 (column 3)] = $0.0328\ M^{-1}\ sec^{-1}$.

[g] Since HAP bound DNA was used as driver, no free single strands participate in the tracer reaction. Thus, as in note [f], only particle single strands need be considered to calculate $K_{T(exp)}$ (see notes [d] and [e]). K_D is first calculated using L_R, the average particle single strand length. $K_{T(exp)} = 0.393\ M^{-1}\ sec^{-1}$ (column 6) × 0.39 (column 5) × [160 (column 3)/600 (column 2)]$^{1/2}$ × [300/160 (column 3)] = $0.148\ M^{-1}\ sec^{-1}$.

[h] Observed tracer rates were calculated by using the equation

$$\frac{T}{T_0} = \exp\{K_T[1 - (1 + K_D C_0 t)^{1-n}]/K_D(1 - n)\} \qquad [3]$$

where T is unhybridized tracer concentration, T_0 is starting tracer concentration, K_T is the rate of tracer-driver reaction, and other symbols are as in Eqs. 1 and 2. K_T is different from K_D since $L_T \neq L_D$. Eq. 3 is obtained by integrating the equation $dT/dt = -K_T ST$, where S is total single strand concentration (Eq. 2). The derivation of the equation is given in Davidson *et al.* (6). Eq. 3 does not include the effects of the particle single strand inhibition noted in this paper. However, the rates obtained in this experiment are very insensitive to the exact form used to fit the data, and for this case even second order fits (Eq. 1) give closely equivalent results. For the calculations in this column, the values of K_D are those calculated in notes [d], [e], and [f]. The listed values of $K_{T(obs)}$ are the best least squares fits to Eq. 3.

bath and the reaction was terminated by freezing the reaction mixture in a dry ice-acetone bath.

Each incubation sample was divided, and a portion of the sample was immediately assayed for DNA fragments containing duplex regions by passage over HAP columns at 60° in 0.12 M PB–0.06% sodium dodecyl sulfate. The remainder of the incubation sample was passed over a Sephadex G-200 column equilibrated with 0.3 M NaCl, 0.01 M piperazine-*N*,*N*′-bis(2-ethanesulfonic acid) (Pipes) (Sigma Biochemicals) buffer at pH 6.7. The DNA was quantitatively recovered from the column and conserved for assay of S1 nuclease resistance.

S1 Nuclease Digestion. S1 nuclease from *Aspergillus oryzae* was used (1, 2). All samples for S1 nuclease digestion contained the same enzyme to DNA mass ratio. The ratio was 20 E/D in the units defined by Britten, Graham, Eden,

Painchaud, and Davidson (in preparation) and resulted in maximum digestion of single strand DNA found on duplex containing fragments. These levels were determined empirically by using as an enzyme substrate 750 NT long calf thymus DNA that had been reacted to C_0t 10 and harvested from HAP.

S1 nuclease digestions were carried out in 150 mM NaCl, 5 mM Pipes buffer, 25 mM acetic acid, 1 mM $ZnSO_4$, 25 mM 2-mercaptoethanol. Digestions were at 37° for 45 min at pH 4.4. Reactions were terminated by quenching in an ice bath plus the addition of PB to 0.1 M. Each reaction mixture contained the C_0t 10 calf thymus DNA preparation described above in 40-fold excess over the *E. coli* [^{3}H]DNA. At the termination of the enzyme digestion the reaction mixture was passed over a Sephadex G-100 column. ^{3}H cpm (*E. coli* DNA) and A_{260} (calf thymus DNA) excluded from the G-100 column were scored as S1 nuclease resistant DNA duplex. The inclusion of the calf thymus DNA provided an internal control for enzyme activity.

Data Reduction. A slightly modified version of the least squares curve fitting program described by Britten *et al.* (8) was used to analyze the reassociation measurements. A variety of functions can be inserted in this program, and it is possible to fix individual parameters as needed and vary the others. Best values of the following parameters are usually determined: the size of the kinetic component, or in other words the fraction of the DNA that reassociates at a given rate; the terminal value of the nonreassociating fraction; the rate constant(s); and depending on the function, any additional variables needed to describe the reaction such as the exponent in Eq. 2.

Reassociation reactions have been expressed in terms of "HAP $C_0t_{1/2}$" in this paper except for Fig. 3 and Table 1, where experimental equivalent C_0ts are given. That is, C_0t is expressed as $C_0t/C_0t_{1/2}$. HAP $C_0t_{1/2}$ is the C_0t at which 50% of the DNA is found in fragments bound to HAP. Thus the data have been normalized to a HAP $C_0t_{1/2}$ equal to 1 so that the second order rate constant for the HAP reaction also equals 1. This permits pooling of data from reactions carried out with different DNA fragment lengths.

RESULTS

Empirical Expressions for Analysis of Reassociation Measurements. In 1968 Britten and Kohne (9) introduced the useful and now familiar form

$$C/C_0 = 1/(1 + kC_0t) \qquad [1]$$

to describe second order DNA reassociation reactions. Here C represents the concentration of totally single stranded fragments, C_0 is the total DNA NT or starting single stranded NT concentration, t is time, and k is the empirically observed second order rate constant. As shown originally by Britten and Kohne and later by many other authors the kinetics of reassociation of almost totally nonrepetitive DNAs such as that of *E. coli* follow Eq. 1 when strand pair formation is assayed by HAP binding. Recently Morrow (4) found that when duplex formation is measured by S1 nuclease resistance, the reassociation kinetics of randomly sheared DNA follow a form that can be described by the non-second order form

$$S/C_0 = [1/(1 + kC_0t)]^n \qquad [2]$$

where S is the concentration of S1 nuclease sensitive (i.e., single stranded) DNA NT. From his data Morrow evaluated n as 0.44. This expression of course reduces to Eq. 1 when n = 1. Eq. 2 was derived earlier by Britten and Kohne (9), who also suggested that the value of n should be about 0.5.

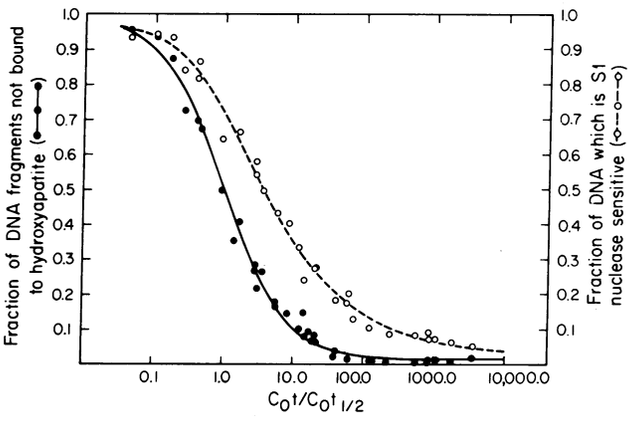

FIG. 1. Reassociation of *E. coli* DNA fragments. DNA duplex formation was assayed on HAP at 60° in 0.12 M PB (●) and by resistance to S1 nuclease digestion (○) (see *Materials and Methods*). Two sets of data were pooled by normalizing both to a HAP $C_0t_{1/2}$ of 1.0 (i.e., $C_0t/C_0t_{1/2}$). The solid line represents a computer least squares solution for reassociation assayed by HAP binding, according to Eq. 1 in *text*. The dashed line represents the best least squares solution for the S1 nuclease kinetics, according to Eq. 2 in *text*. The normalized rate constant K is of course 1.0 for both curves, and the best value of n for the S1 nuclease data is 0.453.

Kinetics of *E. coli* DNA Reassociation Measured by S1 Nuclease Resistance and by HAP Binding. Samples of sheared, denatured *E. coli* DNA were allowed to renature as described in *Materials and Methods*, and at appropriate C_0t values the reactions were stopped by quick freezing in dry ice-acetone. The samples were later thawed, diluted, and divided into two portions. One of these was assayed for S1 nuclease resistance and the other was passed over a HAP column. The results of this experiment are shown in Fig. 1. As expected, the kinetics of appearance of HAP binding follow a second order form. In Fig. 1 the HAP data are fit with Eq. 1, using the least squares procedure referred to in *Materials and Methods*. The kinetics of appearance of S1 nuclease resistance are fit with Eq. 2. Our data follow the form of Eq. 2 reasonably well, though the unavoidable scatter reduces the accuracy of the comparison. Least squares analysis shows that the best value for n of Eq. 2 is 0.45. These results are therefore in excellent quantitative agreement with those reported by Morrow (4) for randomly sheared simian virus 40 DNA.

Fraction of Duplex-Containing Fragments That Remain Single Stranded. The renaturation begins with a first collision and nucleation, the result of which is the formation of a length of duplex equal to the overlap between the strands. In later collisions the single stranded regions become filled in as successive nucleation events occur. Thus the fraction of DNA that is S1 nuclease sensitive progressively decreases. Fig. 1 shows that about 94% of the DNA becomes S1 nuclease resistant when the reaction approaches completion (the remaining 6% may be contaminated with unreactable DNA fragments and is not necessarily the true terminal value). The *initial value* for the fraction of HAP bound fragments that is S1 nuclease insensitive should equal the average amount of overlap that occurs when two fragments containing complementary sequence collide, since early in the reaction only first collision products are present in the duplex fraction. We define the parameter α as the sequence paired per nucleation event (i.e., the overlap), expressed as a

fraction of the single strand fragment length. For fragments terminating at the same place in the sequence, for example, restriction enzyme fragments or whole genomes, $\alpha = 1.0$. Wetmur and Davidson (10) calculated that for an ideal case of randomly sheared fragments of uniform length, $\alpha = 0.66$. In other words two-thirds of each fragment would be included in duplex in an average collision between such fragments. We have confirmed this result using an independent method of calculation and have extended it to the case of a range of fragment lengths. The effect of a distribution of fragment lengths is to decrease the value of α. For fragment length distributions roughly similar to that of the randomly sheared DNAs used in this study [as measured in the electron microscope (see *Materials and Methods*)], a computer simulation provides values for α in the range 0.55 to 0.6 (this simulation will be described in paper no. 2 of this series†). The exact value depends on the fragment length distribution imposed.

Theoretically the value of α could be obtained directly by measuring the S1 nuclease sensitivity of HAP bound DNA early in the reaction. In practice this is difficult with fragment lengths of the range used in this study. Quantitative elution of the reassociated DNA (i.e., with 0.5 M PB) cannot always be achieved and strand scission may occur. Hence our best empirical estimate of α is derived from the data of Fig. 1. The fraction S1 resistant (R) and the fraction HAP bound (B) were determined on aliquots, and α was calculated as $(B - R)/B$. The difference is plotted in Fig. 2. Earlier than 0.5× the $C_0t_{1/2}$ the difference between the two measurements is too small in absolute terms to be estimated reliably, and thus the ordinate intercept in Fig. 2 is set at this point in the reaction. Here about 33% of the DNA is bound to HAP, but only about 18% is S1 nuclease resistant. However, at 0.5× the $C_0t_{1/2}$ almost none of the duplex containing particles include more than two fragments. That is, these particles are mainly the products of first collisions. Fig. 2 shows that the fraction of bound fragments that is S1 nuclease sensitive at relatively low C_0t is about 0.45. The value of α obtained from these data is then (1–0.45) or 0.55. This result is consistent with the value calculated on a statistical basis in the computer simulation, as noted above.

At this point a contradiction is apparent between the best estimates of α (0.55–0.6), and the value of the exponent n in Eq. 2 fit to the S1 nuclease data (0.45). For the initial few percent of the reaction n should equal α‡. It follows that Eq. 2 when $n = 0.45$ is an erroneous form for the early part of the reaction, and more importantly, that there is no simple physical meaning for the measured value of n. We show in the second paper of this series† that several complex factors are indeed involved. For the moment we retain Eq. 2 as an adequate empirical description of single strand remaining for most, if not all, of the course of the reaction.

Fig. 2 shows that as the reaction proceeds the fraction of the bound fragments that are S1 nuclease sensitive decreases from the maximum value of $(1 - \alpha)$. Since this is mainly the result of successive nucleations on the same fragments, the expected consequence is the buildup of large hyperpolymers

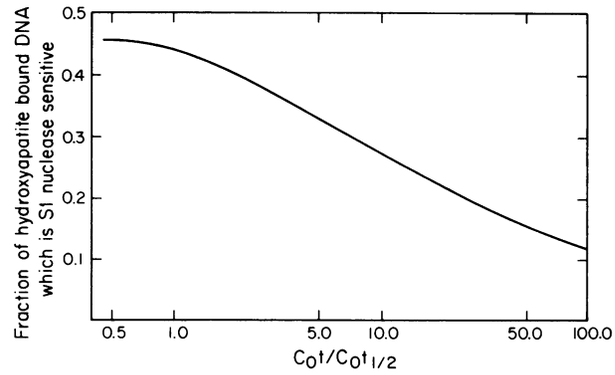

FIG. 2. Fraction of HAP bound DNA that remains single stranded as a function of C_0t. The fraction of HAP bound DNA that is S1 nuclease sensitive was calculated from the computer least squares fits shown in Fig. 1. At each C_0t the fraction of the total DNA in fragments bearing duplex was determined from the curve describing the HAP kinetics in Fig. 1 (HAP bound fraction). At the same C_0t the fraction of the total DNA that was S1 nuclease resistant was calculated from the line describing the S1 nuclease kinetics (S1 resistant duplex). The fraction of HAP bound DNA that is single stranded at this C_0t is therefore [1-(S1 resistant duplex/HAP bound fraction)]. A series of such calculations were made from approximately one-half the normalized HAP $C_0t_{1/2}$ (i.e., C_0t 0.5) to 100 times the $C_0t_{1/2}$.

late in the reaction. These have been observed both in the electron microscope and by physical-chemical means, and will be the subject of a separate report (Graham, Eden, Davidson, and Britten, in preparation).

Rate of Reaction of Particle Single Strands with Free Single Strands. In an S1 nuclease kinetic measurement much of the reaction observed is between the single stranded regions of particles and other single stranded elements. It is possible that the duplex regions of the particles inhibit nucleation with the adjoining single stranded regions, so that the rate of their reaction is less than that of free single strands of comparable length. To test this possibility the following experiments were carried out. *E. coli* DNA was allowed to renature to a point where almost all the DNA was present in particles, as judged by its binding to HAP. In one case the particles were physically isolated from the small amount of free single stranded DNA remaining. Trace amounts of labeled single stranded DNA were then added and the rate of reaction of the tracer with the already renatured driver DNA was measured. The quantity of particle single strands in the driver DNA preparation at the point of addition of the tracer was estimated as its S1 nuclease sensitivity. Details of three such experiments are presented in Table 1, and an example of the kinetics obtained is presented in Fig. 3. Here the reassociation kinetics of the driver DNA are shown, and in addition the tracer DNA reactions beginning at driver DNA C_0ts 20 and 1000 are illustrated.

The results of these experiments are presented in Table 1. An expected rate for the tracer-particle single strand reaction is calculated, on the basis that particle single strands of given length are as effective in initiating nucleation as if they were free single strands of the same length and at the same concentration (column 7, Table 1). When the observed tracer rate (column 8, Table 1) is compared to this calculated free strand rate it is found that the observed tracer rate is about half the calculated rate (column 9, Table 1). That is, the tracer reacts at about half the rate it would have had if added to a single strand driver of equivalent length at the beginning of the reaction. This provides a direct measure of

† R. J. Britten and E. H. Davidson, *Proc. Nat. Acad. Sci. USA.*, to be submitted.

‡ This can be shown in the following way. From Eq. 1, the initial rate of disappearance of single strands is $dC/dt = -kC_0^2$. Thus for the first collision case the disappearance of single stranded DNA NT should be $dS/dt = -\alpha kC_0^2$. On the other hand, from Eq. 2, $dS/dt = -nkC_0^2(1 + kC_0t)^{-1-n}$. Early in the reaction when t is small, $dS/dt = -nkC_0^2$. Therefore, early in the reaction, $\alpha = n$.

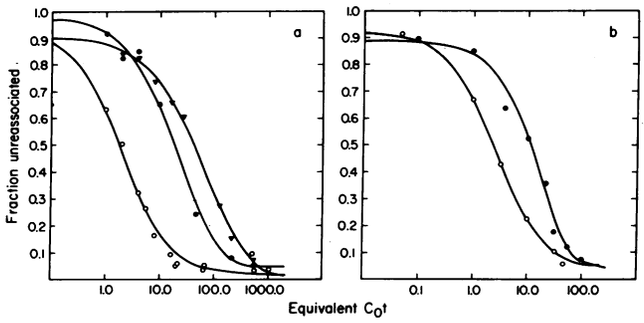

FIG. 3. Tracer DNA reaction with particle single strands. (a) O, Reassociation of 1000 NT *E. coli* DNA measured by the portion of A_{260} bound to HAP ("driver DNA"). The best least squares fits are shown by the lines. ●, Reaction of 300 NT [^{3}H]DNA fragments added to the driver at C_0t 0 assayed by hydroxyapatite. ▼, Reaction of the same tracer added to the driver DNA in a separate series of samples when the driver DNA has attained C_0t 1000. At this stage 6.4% of the driver DNA is single stranded (Table 1). For comparison the latter two curves are plotted together with the driver DNA curve by regarding the time of tracer addition as C_0t 0 and the single strand concentration at the time of tracer addition as C_0. The calculated rates are shown in Table 1. (b) O, Reassociation of 600 NT *E. coli* DNA measured by HAP binding as in (a). At C_0t 20 the fraction that binds was isolated and reacted with the 300 NT long tracer DNA. ●, Reassociation of the tracer with this particle driver. The calculated rates of these reactions are shown in Table 1. As above, the single strand driver concentration at time of tracer addition is taken as C_0 to permit a comparable plot.

the relative inhibition in the reaction of free single strands with particle single strands. The measurement is not of very high accuracy because of the several quantitative assumptions required in carrying out the appropriate calculations (see notes to Table 1). However, we believe it to be reliable with an error of about ±20%. This calculation provides an estimate of inhibition *after* the best length correction possible at our present stage of knowledge has been made. The roughly 2-fold inhibition observed is therefore due to some factor other than progressive shortening of the driver particle single strands. We stress that these measurements provide only an average, or overall estimate of the inhibition, which is likely to change during the course of the reaction as a function of strand length, size of particle, and possibly other factors such as ionic strength and temperature as well.

DISCUSSION

Calculation of HAP and S1 Nuclease Renaturation Kinetics. It is clear from the foregoing that neither the traditional second order expression (Eq. 1) nor the modified forms proposed by Britten and Kohne (9) and Morrow (4) (Eq. 2) provide a description of renaturation kinetics that is satisfactory from a mechanistic point of view. That is, no simple physical meaning can be associated with n of Eq. 2. Both Eqs. 1 and 2 are of course empirically useful for purposes of data reduction. However, neither explicitly takes into account reactions other than the initial nucleation of two free single strands. Furthermore, the experiments described in Fig. 3 and Table 1 provide direct evidence that nucleation rate is diminished in reactions involving single stranded regions of particles compared to equivalent reactions between free single strands.

There are two classes of explanation for the overall retardation revealed by these investigations. One of these we shall term "length effects" and the other "particle inhibition." By "length effects" we denote the decrease in the rate of successful nucleation between single strands which is due simply to the progressive shortening of the single stranded regions. The expected "length effects" for single strands on particles is assumed to be the same as for free single strands. We use the term "particle inhibition" to denote decrease in the per NT reactability (i.e., per NT nucleation probability) of single strands which belong to duplex-containing particles, due to the presence of the duplex. For example, one could imagine the rigid duplex regions affecting the excluded volume parameters of neighboring single stranded regions. Particle inhibition is measured directly in the experiments of Table 1, and was estimated to result in about 2-fold rate decrease for reactions in which particles participate. We show in the second paper of this series† that both length effects and particle inhibition are required to account for the observed kinetics.

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