On the Processive Mechanism of Escherichia coli DNA Polymerase I*

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A procedure has been developed to assess whether polymerization of nucleotides by DNA polymerases is processive, that is, whether a succession of polymerization steps occurs without release of the enzyme from the template. The method involves measurement of the ratio of deoxyguanylate to deoxycytidylate incorporated in the course of replicating a segment of the right hand cohesive end of phage λDNA with the sequence 5' G C G C C C G C C G 3'. In the case of Escherichia coli DNA polymerase I, each enzyme molecule completes synthesis of the sequence before dissociation occurs. Furthermore, at both 6 and 22°, the polymerase remains bound to the λDNA template after synthesis has been completed.

Template challenge experiments, in which the polymerase is allowed to begin synthesis in the presence of a molar excess of λDNA before addition of a very large excess of calf thymus DNA, show that under the conditions used, productive binding of polymerase to λDNA is a slow process requiring 1 to 2 hours. After synthesis has been completed, polymerase remains bound to the λDNA in spite of the availability of new primer termini.

The association, polymerization, and dissociation rates measured in these experiments suggest that the polymerization reaction catalyzed by DNA polymerase I is processive, and that hundreds of nucleotides may be polymerized between each association and dissociation.

DNA polymerase I of Escherichia coli has been the most thoroughly investigated of all the known DNA polymerases, and as a consequence, a great deal is known about its active site (1-4). However, an unanswered question regarding its mechanism is whether the polymerization of nucleotides proceeds in a processive or nonprocessive manner. The reaction could be nonprocessive so that dissociation of the polymerase from the primer-template occurs following addition of each nucleotide, or it could be processive, with many nucleotides incorporated before the enzyme dissociates. A partially processive mechanism is also possible, such that a small number of nucleotides are incorporated between each association and dissociation.

To answer this question, we have investigated the filling in of the cohesive ends of phage λDNA by E. coli DNA polymerase I. With this DNA as primer-template, a known sequence of nucleotides is incorporated into the cohesive ends, and furthermore, the composition of the nucleotides incorporated changes in a predictable way as polymerization proceeds (Scheme 1) (5, 6).

When dGTP and dCTP are provided as substrates, only those nucleotides enclosed by the bracket are incorporated into the right hand end, and there is no synthesis at the left hand cohesive end. If nucleotide incorporation occurs by a processive mechanism, at a low molar concentration of enzyme relative to DNA, the ratio of dG to dC incorporated will remain constant and will not change with the extent of reaction. On the other hand, if the mechanism is nonprocessive, early in the reaction a large proportion of the λDNA molecules will have incorporated only 1 or 2 dG residues into their right hand cohesive end, and the ratio of dG to dC will be high. As the reaction proceeds to completion, the dG/dC ratio will drop to a value of 3, the ratio of the number of dG and dC residues incorporated into the right hand cohesive end.

This type of analysis, when applied to E. coli DNA polymerase I, has demonstrated clearly that polymerization proceeds by a processive mechanism.

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THEORY

For reactions in which only dGTP and dCTP are substrates, the average ratio of dG to dC incorporated per right hand cohesive end can be predicted for different mechanisms of polymerization from (a) the average number of enzyme-DNA associations per cohesive end, (b) the average number of nucleotides incorporated per association, and (c) the sequence of nucleotides incorporated into the right hand cohesive end. The only assumption made is that the time required to polymerize the average number of nucleotides per association is negligible. No assumptions need be made concerning the rate of association or dissociation of enzyme and primer-template. A practical consideration for purposes of experimental design is that sufficient incorporation occurs to permit reliable measurements. Only a brief summary of the pertinent calculations is given here. A detailed analysis is presented in the Appendix.

Case 1—The average number of nucleotides incorporated per association is many times larger than the length of the λ cohesive ends (processive polymerization). In this case, association occurs, the entire octanucleotide sequence \( \text{G-G-G-G-C-G-G-C-G} \) is synthesized before dissociation occurs. This results in a dG/dC ratio of 3 for any number of binding events, and thus for any level of incorporation (Fig. 1).

Case 2—The enzyme dissociates after association and addition of a single nucleotide (nonprocessive polymerization). In this case, the dG/dC ratio depends on the distribution of association events over the population of right hand cohesive ends. At very low levels of incorporation, the number of associations per cohesive end will be low. A very large dG/dC ratio will then result because the first three nucleotides incorporated on each right hand cohesive end are dG. At high levels of incorporation, when most right hand cohesive ends are nearly filled, the dG/dC ratio will approach 2. The expected change in ratio is shown as a rapidly decaying function in the curve labeled 1 only in Fig. 1.

Case 3—The enzyme dissociates after addition of a few nucleotides (partially processive). If, for example, dissociation occurs after addition of an average of two nucleotides, the change in the dG/dC ratio will be a decaying function which begins at a lower value than in Case 2, but also decays to a value of 3. If dissociation occurs after addition of an average of three nucleotides, the function begins at a still lower value and again decays to 3.

As seen in Fig. 1, the dG/dC ratio is a very accurate indicator of the average number of nucleotides incorporated per association up to levels of about 25% of total incorporation.

INTACTNESS OF X\(\alpha\)DNA—Even small numbers of nicks or gaps in the X\(\alpha\)DNA might affect significantly the extent of nucleotide incorporation. The intactness of the X\(\alpha\)DNA was tested by measuring the extent of incorporation of the four deoxynucleotides using a 7-fold excess of enzyme over DNA molecules at 6\(^\circ\), and a total salt concentration of 180 mM. Under these conditions, filling in of the cohesive ends of X\(\alpha\)DNA by DNA polymerase I was performed as described by Wu et al. (7); modifications are described in the legends to the figures and tables.

Concentrations of DNA are expressed as moles of duplex DNA molecules unless stated otherwise.

MATERIALS AND METHODS

Bacteriophage X\(\alpha\)DNA was isolated according to the method of Wu et al. (7). Activated calf thymus and X\(\alpha\)DNAs were prepared by limited digestion with pancreatic deoxyribonuclease (8). The total number of termini was determined by treating the DNA sequentially with \(E\). coli alkaline phosphatase and polynucleotide kinase in the presence of [\(\gamma\)-\(32\)P]ATP, as described by Richardson (9). The \(E\). coli DNA polymerase I used was Fraction VII (10). \([\alpha\)-\(32\)P]dCTP (60 to 100 Ci/mmol) and \([\alpha\)-\(32\)P]dGTP (20 Ci/mmol) were purchased from New England Nuclear Corp. The four unlabeled deoxynucleoside triphosphates were purchased from P-L Biochemicals.

Filling in of the cohesive ends of X\(\alpha\)DNA by DNA polymerase I was performed as described by Wu et al. (7); modifications are described in the legends to the figures and tables.

RESULTS

Incorporation of both [\(\alpha\)]dGMP and [\(\beta\)]dCMP reached a maximum within 2 hours and remained at this level for 8 hours indicating that nucleotide incorporation occurred only into the cohesive ends (Fig. 2). The level of enzyme used was sufficient to catalyze maximum synthesis; doubling the enzyme concentration did not cause a significant increase in either the rate or extent of incorporation.

From the known dodecanucleotide sequences of the cohesive ends of X\(\alpha\)DNA, a total of 10 dG and 10 dC residues should be incorporated into each molecule. These values should therefore permit a precise determination of the specific radioactivities of the [\(\alpha\)]dGTP and [\(\beta\)]dCTP used as substrates. However, under conditions where complete filling in of the cohesive ends should occur, incorporation of dC was somewhat variable, suggesting that the 10th dC may not have been incorporated into every X\(\alpha\)DNA molecule. To circumvent this difficulty, we have used the [\(\alpha\)]dGMP incorporated into the cohesive ends...
to give the standard value of 10 dG residues/ADNA molecule. The specific activity of the labeled dCTP was then determined by comparing the incorporation of dG and dC into activated λ- and calf thymus DNA. Using the specific activity of \([\alpha^{-32P}]dCTP\) obtained by this method, we calculate from the experiment shown in Fig. 2 that 9.3 dC residues had been incorporated per λDNA molecule. The specific radioactivities determined in this way have been used in all subsequent experiments to determine the number of labeled nucleotides incorporated per DNA molecule.

An experiment similar to that shown in Fig. 2 was performed with the exception that only dGTP and dCTP were added. Incorporation also reached a plateau, with 6.4 dG and 2.4 dC residues incorporated per λDNA molecule (Fig. 3), values that agree well with the 6 to 7 dG and 2 dC residues expected.1

Incorporation of dG and dC into λ Cohesive Ends at 6° in Presence of Limiting Enzyme—The incorporation of dG and dC was determined at a molar ratio of 3'-termini to polymerase of 9 (Fig. 4). Under these conditions, the extent of incorporation at various times ranged up to about 6% of the ADNA molecule. At very low levels of incorporation (about 2%) the dG/dC ratio was approximately 3. As incorporation rose to a 5-fold higher level, there was no measurable drop in ratio; in fact, it increased from 2.9 to about 3.4. The slight rise is most likely caused by the slow exchange of labeled dG with the unlabeled dG at the left hand 3'-terminus (Scheme 1). This result shows that the mechanism of polymerization by DNA polymerase I is processive. Indeed, if the enzyme were functioning nonprocessively, the level of incorporation would be expected to increase slowly under these conditions until the complete octanucleotide sequence had been synthesized on every molecule. This was not observed; instead, incorporation leveled off at about 6% of maximum after 2 to 3 hours. The plateau most probably represents a situation in which each enzyme molecule has filled in a cohesive end but cannot dissociate and continue further polymerization on other λDNA molecules. As shown in Table I, the plateau value reached at limiting enzyme concentrations was, in fact, proportional to enzyme concentration. It is also clear that fewer ends were repaired than the number of enzyme molecules added, suggesting that approximately 1/3 of the DNA polymerase molecules were inactive under the conditions of the experiment.

Incorporation of dG and dC into λ Cohesive Ends at 6° in Presence of Excess Enzyme—To be certain that a change in ratio can be detected easily, and to determine the rate of nucleotide polymerization onto individual DNA molecules, incorporation of dG and dC was measured at very short intervals after the addition of a 60-fold excess of enzyme over 3'-termini. As shown in Fig. 5, dG incorporation began immediately and increased rapidly, but incorporation of dC began slowly relative to dG and accelerated after a delay of about 15 to 30 s. The dG/dC ratio, starting at above 17 at 10 s, dropped to 3 by 5 min. Clearly, changes in ratio can be easily and accurately measured. These data further indicate that polymerization on a given molecule is a rapid event, completed within 1 to 2 min. As judged by the kinetics of nucleotide incorporation in the presence of limiting enzyme (Fig. 4), it appears that a binding or initiation step prior to the first polymerization event must be a much slower process than the polymerization itself, with a half time of 1 to 2 hours at low concentrations of polymerase.

Restart of Synthesis after Termination at 6°—It is possible that incorporation reached a plateau at low enzyme levels because of inactivation of the polymerase rather than because of the slow rate of dissociation of the enzyme from the primer-template. As shown in the experiment described in Fig. 6, this is not so. The reaction was begun with only \([\alpha^{-32P}]dCTP\) and \([\alpha^{-32P}]dCTP\) in the presence of a 6-fold excess of 3'-termini over polymerase. As expected, incorporation reached a plateau at approximately 2 hours. However, when unlabeled dATP and dTTP were added at 4 hours, polymerization promptly resumed. This result shows clearly that the enzyme remained active. Furthermore, the increment of further incorporation is consistent with the completion of synthesis on those ends to which enzyme molecules were already bound.3 Presumably, the overall polymerization rate decreases and incorporation ultimately stops because enzyme molecules bound to the DNA after the completion of synthesis cannot dissociate easily and bind new DNA termini.

Incorporation of dG and dC into Cohesive Ends of λDNA at

1The dG incorporation should range from 6 to 7 because the 3'-terminal guanylate on the left hand end of the ADNA molecule can exchange partially with \([\alpha^{-32P}]dCTP\) in the reaction mixture, presumably as a result of the concerted action of the 3'-5' exoexonuclease and polymerase activity of DNA polymerase I (10, 11).

2This finding agrees with the observation made by Wu that the complete filling in of the cohesive ends of λDNA does not occur in the presence of limiting amounts of DNA polymerase I (R. Wu, personal communication).

3The expected values are 11 dG and approximately 9.5 dC residues per DNA molecule. These levels take into account exchange of \([\alpha^{-32P}]dGMP\) with the terminal 3'-dGMP at the left hand cohesive end (11, 12) and the incomplete insertion of the terminal dC at the right cohesive end (cf. Scheme 1).
TABLE I

<table>
<thead>
<tr>
<th>DNA polymerase I</th>
<th>dG incorporation</th>
<th>Average number of λ cohesive ends filled in per polymerase molecule</th>
</tr>
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<tbody>
<tr>
<td>fmol</td>
<td>fmol</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>21</td>
<td>0.60</td>
</tr>
<tr>
<td>33</td>
<td>56</td>
<td>0.49</td>
</tr>
<tr>
<td>100</td>
<td>161</td>
<td>0.47</td>
</tr>
</tbody>
</table>

Effect of DNA polymerase I concentration on extent of synthesis at 6°

Reaction mixtures were the same as those described in the legend to Fig. 4. After 10 hours of incubation at 6° in the presence of the amounts of DNA polymerase I indicated, aliquots (30 μl) were removed to determine nucleotide incorporation. The specific activity of the [3H]dGTP was 34 cpm/fmol. Extents of dG incorporation are given for the original volume of reaction mixture, containing 150 fmol of DNA. Assuming that enzyme molecules distribute evenly between left and right hand cohesive ends, and that a total of 7 dG residues can be incorporated per λDNA molecule, 1 then each polymerase molecule can incorporate an average of 3.5 dG residues per end. Using this value, the average number of ends filled in was calculated from the extent of dG incorporation. Dividing the average number of ends filled by the number of polymerase molecules yields the average number of ends filled in per enzyme molecule.

15 and 22°—To determine whether DNA polymerase I acts processively at temperatures higher than 6°, the time course of nucleotide incorporation in the presence of limiting enzyme was examined at 15 and 22°. Filling in of λDNA cohesive ends at 22° is shown in Fig. 7. When only dGTP and dCTP were added, the dG/dC incorporation ratio remained near 3 at all times. Thus, polymerization is processive at 22° as well as at 6°. In the presence of all four deoxynucleoside triphosphates, the rate of incorporation leveled off, indicating that the dissociation of polymerase from DNA is still very low at 22°. However, the leveling off was not as complete at 22° as at 6° (compare incorporation profiles and time scales in Figs. 4 and 7). This suggests that dissociation of the enzyme from filled cohesive ends and binding to unfilled ends occurs somewhat more rapidly at the higher temperature. Incorporation at 15° was qualitatively similar to that at 6 and 22°, with the incorporation rate after leveling off intermediate between that at 6 and 22° (data not shown).

As shown in Table II, the extent of incorporation increased
with temperature. This finding is consistent with the apparently greater dissociation rate at higher temperatures. Indeed, at 22°, 1.5 cohesive ends were filled per polymerase molecule, compared with 0.5 at 6°.

To determine whether synthesis could resume after having leveled off at temperatures higher than 6°, an experiment similar to that described in Fig. 6 was performed at 22°. As shown in Fig. 7, addition of dATP and dTTP, after incorporation of labeled dG and dC had essentially ceased (1.5 hours) resulted in further incorporation of dC and dG. The increment in incorporation is consistent with completion of synthesis at these ends into which dC and dG had been incorporated initially, again suggesting that the enzyme dissociates slowly from partially filled ends, even at 22°.

When addition of dATP and dTTP was delayed until 3 or 4 hours after the start of synthesis, the increment in additional dC and dG incorporation was substantially less than that required for completion of all ends which had been partially filled (data not shown). This finding is consistent with substantial dissociation of enzyme from partially filled ends by 3 to 4 hours at 22°. Inasmuch as some partially filled ends lack bound polymerase, additional synthesis at these ends cannot occur.

**Competition between λ- and Calf Thymus DNAs for DNA Polymerase I—**The results presented thus far suggest that the dissociation of DNA polymerase I from λDNA is a very slow process. If so, then addition of a large excess of a heterologous DNA (for example, activated calf thymus DNA) to a reaction in which a limiting number of polymerase molecules have completed incorporation on λDNA should not produce further

![Fig. 6. Restart of synthesis after termination at 6°. A reaction mixture (340 µl) was prepared as described in the legend to Fig. 2, except that dATP and dTTP were omitted. The mixture was made 120 mM in NaCl to further inhibit incorporation into the internal regions of the duplex λDNA (5). The specific activities of the deoxynucleoside triphosphates were 38 cpm/fmol for the [3H]dGTP and 113 cpm/fmol for the [3H]dCTP. The reaction was started by the addition of 100 fmol of DNA polymerase I in 2 µl of polymerase diluent and incubated at 6°. At 4 hours (arrow), dATP and dTTP were added (to a final concentration of 1 µM each) to an aliquot (100 µl), and incubation of both the original mixture (circles) and supplemented aliquot (squares) was continued. Samples (20 µl) were removed at the times indicated, and acid-insoluble radioactivity was determined. A relative scale of nucleotide incorporation per λDNA molecule is indicated on the right hand ordinate, based on the incorporation of approximately 6.5 dG and 4.5 dC residues in the presence of dGTP and dCTP only.

The values indicated represent the amount of nucleotide incorporated per 170 µl of reaction mixture containing 150 fmol of DNA.

![Fig. 7. Restart of synthesis after termination at 22°. Reaction mixtures (340 µl) were prepared as described in the legend to Fig. 2, except that the mixtures were made 120 mM in NaCl. Reactions were started by addition of 100 fmol of DNA polymerase I in 1 µl of polymerase diluent and incubated at 22°. The specific activities of the deoxynucleoside triphosphates were 19.9 cpm/fmol for the [3H]dGTP and 36.2 cpm/fmol for the [3H]dCTP. One mixture (squares) was made 10 µM each in dATP and dTTP. The second mixture (circles) received no dATP or dTTP. The third mixture (triangles) received no dATP or dTTP until 1.5 hours, at which time it was made 10 µM each in dATP and dTTP. Aliquots (30 µl) were removed after 6 and 9 hours of incubation for measurement of nucleotide incorporation. The values for dG incorporation represent the average of these two time points from the plateau region; they are expressed as femtomoles of dG incorporated per λDNA molecule. The average number of λDNA molecules incorporated per reaction mixture volume containing 150 fmol of λDNA.

**TABLE II**

<table>
<thead>
<tr>
<th>Temperature</th>
<th>dG incorporation (fmol)</th>
<th>Average number of λDNA molecules per enzyme molecule</th>
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<tbody>
<tr>
<td>6°</td>
<td>121</td>
<td>0.48</td>
</tr>
<tr>
<td>15°</td>
<td>177</td>
<td>0.71</td>
</tr>
<tr>
<td>22°</td>
<td>375</td>
<td>1.50</td>
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</table>
DNA synthesis. To test this idea, the experiment described in Fig. 8 was performed. Reaction mixtures containing either λDNA alone or λDNA in addition to activated calf thymus DNA were prepared. Polymerization began rapidly, but, as expected, leveled off in 1 to 2 hours in the mixture containing only λDNA. In contrast, in the mixture containing both λDNA and activated calf thymus DNA, rapid incorporation continued throughout the experiment. Addition of activated calf thymus DNA to 1 aliquot of the mixture containing only λDNA at 1/2 hour, a time before the plateau was reached, allowed continued incorporation at a rate slightly lower than that in the mixture that contained both DNA species initially. This result suggests that only a small fraction of the total polymerase was sequestered by λDNA; such molecules are presumably unavailable for synthesis on newly provided primer-template. When activated calf thymus DNA was added to a 2nd aliquot of the mixture containing only λDNA at 4 hours, a time when plateau had been reached, further incorporation occurred at an extremely low rate. This result suggests that most of the DNA polymerase I molecules were sequestered by λDNA after a plateau of incorporation had been reached.

**DISCUSSION**

Our results indicate clearly that *Escherichia coli* DNA polymerase I catalyzes the polymerization of nucleotides by a processive mechanism. Using the cohesive ends of λDNA as a primer-template, we have demonstrated that polymerization is processive for 12 nucleotides and probably many more at both 6 and 22°C. We have also shown that after completion of synthesis, the polymerase remains tightly bound to the extended 3'-terminus of the newly synthesized DNA. This result agrees with the findings of England et al. (10) who showed that DNA polymerase can be effectively sequestered from solution by the addition of DNA.

The fact that the reaction usually requires 1 to 2 hours to reach a plateau at 6°C under conditions of limiting enzyme, while polymerization of eight nucleotides can occur in about 2 min in the presence of excess enzyme, suggests the following mechanism for the filling in of the cohesive ends of λDNA by DNA polymerase I. A relatively long interval (1 to 2 hours) is required to find and correctly bind a primer and initiate polymerization. This interval is given by the time required to reach a plateau when enzyme is limiting (Fig. 4). Polymerization of the eight nucleotides then occurs rapidly, with a half-time of approximately 1 min (Fig. 5). The final step, the dissociation of the polymerase from the extended 3'-terminus, is the slowest. As judged by the very slow rise in plateau level, this process has a half-time of approximately 10 to 20 hours. If polymerization is allowed to occur for 10 hours at a rate of four nucleotides per min, and if the dissociation rate is not greatly increased by continuous polymerization, an average of 2400 nucleotides would be incorporated between each association and dissociation event.

These results suggest that there is physical translocation of the DNA polymerase along a DNA strand during repair or synthesis. Such a translocation is analogous to the translocation of the ribosome over mRNA in the course of protein synthesis (14) and to the translocation of RNA polymerase along DNA during transcription (15), and may prove to be a universal mechanism for the efficient transfer of sequence information from one macromolecule to another.

Both initiation and termination of transcription by RNA polymerase appear to be under strict control involving specific nucleotide sequences and RNA polymerase-subunit interactions which define the sites of initiation and termination (16). It is likely that interaction of DNA polymerase I with other proteins or perhaps even with specific sequences will also affect its dissociation from DNA. Clearly, if DNA polymerase I fills in gaps in vivo, it must dissociate before DNA ligase can effectively bind and seal the nick. *In vitro* experiments have, in fact, shown that DNA ligase and RNA polymerase both compete with DNA polymerase I for binding sites on DNA (17, 18). Such interactions might possibly displace bound DNA polymerase I in vivo.

McClure and Jovin (19) have reported that within the time required for the turnover of one nucleotide at 4°C, activated calf thymus DNA can compete effectively with d(A-T) co-polymer onto which nucleotides are being polymerized by DNA polymerase I. However, their experiments depend upon measurements of the affinity of the enzyme for polynucleotides whose structures are not well defined.

Chang (20) has also reported that *E. coli* DNA polymerase I can switch to a competing template in the process of synthesis. However, these experiments were performed at 35°C with a very low time resolution, so that many nucleotides might well have been incorporated into one primer-template before dissociation occurred.
Our measurements of processivity do not depend upon template challenge experiments. Instead they take advantage of a well defined primer-template in which the sequence of nucleotides is known. Under the conditions that we have used, the three major steps in the polymerase-catalyzed reaction can be monitored quantitatively: association, polymerization, and finally, dissociation.

On the other hand, our results taken together with those of McClure and Jovin (19) and of Chang (20), do suggest that DNA polymerase I may not be processive under all conditions. In particular, our finding that the rate at which the enzyme dissociates from the primer-template increases with increasing temperatures indicates that the number of nucleotides polymerized between each association and dissociation is likely to depend not only on temperature, but possibly on pH, salt, and other conditions of the reaction.

APPENDIX

This section describes the detailed calculation of the dG/dC incorporation ratios shown in Fig. 1.

Case 1—The enzyme fills in completely the cohesive end after each association (processive polymerization). Let n represent the average number of associations per right hand cohesive end of ADNA, where no end may undergo more than one association. The average number of dG and dC residues incorporated per right hand cohesive end (given by G_s and C_s, respectively) are then represented by the equations:

\[ G_n = 6n \quad (1) \]
\[ C_n = 2n \quad (2) \]

for any n from zero to one. Thus, it is evident that the dG/dC ratio is 3 for any value of n as denoted by the line labeled 1 in Fig. 3.

Case 2—The enzyme dissociates after association and addition of one nucleotide (nonprocessive polymerization). The expected dG/dC ratio can be calculated by use of the Poisson distribution equation in the form:

\[ P(b,s) = \frac{e^{-n} n^s}{s!} \quad (3) \]

where n is the average number of associations per right hand cohesive end, and s is the number of associations being considered per end for each calculation. P_s then represents the fraction of all right hand ends undergoing s associations. This is an entirely general formulation which will be recalled in the next case. Because, in this case, each association leads to one nucleotide incorporated, P_s also represents the fraction of right hand cohesive ends which have s residues incorporated. The average number of dG and dC residues polymerized per right hand end is then given by:

\[ G_s = \sum_{b=1}^{\infty} G(b) P(b,s) \quad (4) \]
\[ C_s = \sum_{b=1}^{\infty} C(b) P(b,s) \quad (5) \]

where G_s and C_s represent the number of dG and dC residues polymerized per end having s total nucleotides polymerized. The values of G_s and C_s are derived from the known sequence of incorporated nucleotides, 5'-G-G-C-G-C-G-3'. For values of s greater than 8, polymerization is complete so that G_s is 6 and C_s is 2. The dG/dC ratio can now be expressed as

\[ \frac{G_n}{C_n} = \frac{\sum_{s=1}^{\infty} G_s}{\sum_{s=1}^{\infty} C_s} = \frac{\sum_{s=1}^{\infty} G(b) P(b,s)}{\sum_{s=1}^{\infty} C(b) P(b,s)} \quad (6) \]

for any value of n. The corresponding extent of incorporation is then expressed as

\[ \frac{G_n}{C_n} = \frac{1}{8} \sum_{s=1}^{\infty} (G_s + C_s) P_s \quad (7) \]

Because G_s + C_s = s for 1 ≤ s ≤ 8 and G_s + C_s = 8 for s ≥ 8, the extent of incorporation becomes

\[ \frac{G_n}{C_n} = \frac{1}{8} \sum_{s=1}^{8} \left( s \frac{s^n}{s!} \right) + \frac{8}{8} \sum_{s>8} \left( s \frac{s^n}{s!} \right) \quad (8) \]

These formulae have been used to calculate the curve labeled 1 only in Fig. 1.

Case 3—The enzyme dissociates after addition of a few nucleotides (partially processive polymerization). Let us assume that dissociation occurs after an average of m nucleotides are incorporated, where m may be any value. (Values of m larger than eight will not be considered since these produce a situation essentially the same as Case 1).

We must now consider individual subpopulations of right hand ends based on the value s, the number of associations that have occurred. The fractional distribution of the number of nucleotides incorporated for any subpopulation value of s is given as

\[ P_{b,s} = \frac{i^{ms} e^{-ms}}{b^s} \quad (9) \]

where b is the number of incorporations considered per end and m and s are defined above. P_{b,s} then represents the fraction of those ends undergoing s associations which have b nucleotides incorporated. For any particular s value, all P_{b,s} for b ≥ 8 may be summed. This sum represents the fraction of those ends undergoing s associations which have been completely filled.

To obtain the average number of dG and dC residues incorporated, given s associations with m nucleotides incorporated per association, we use the expressions:

\[ G_s = \sum_{b=1}^{\infty} G(b) P(b,s) \quad (10) \]
\[ C_s = \sum_{b=1}^{\infty} C(b) P(b,s) \quad (11) \]

where G_s and C_s are the numbers of dG and dC residues, respectively, which are incorporated when b nucleotides are polymerized. For values of b ≥ 8, G_s is 6 and C_s is 2. G_s and C_s then represent the average number of dG and dC residues incorporated into the subpopulation of ends undergoing s associations. Separating those terms for b ≥ 8, and substituting the formulation of P_{b,s} given in Equation 9 gives:

\[ G_s = \sum_{b=1}^{7} G(b) e^{-ms} + \frac{6}{8} (ms)^b e^{-ms} \quad (12) \]
\[ C_s = \sum_{b=1}^{7} C(b) e^{-ms} + \frac{2}{8} (ms)^b e^{-ms} \quad (13) \]

For any value of n, the average number of associations per right hand cohesive end, we can reformulate Equations 4 and 5 utilizing Equations 3, 12, and 13 to give

\[ \sum_{s=1}^{\infty} s^n e^{-ms} \quad (14) \]
The calculated value of $G_n$ is divided by that for $C_n$ to obtain the $dG/dC$ ratio for any $n$. For the extent of incorporation at any $n$ we use $(G_n + C_n)/8$. These formulae have been used to calculate the functions for various values of $m$ labeled average of 2, 3, etc. in Fig. 1.

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