Macromolecular crowding increases binding of DNA polymerase to DNA: An adaptive effect

(metabolic buffering/excluded volume)

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ABSTRACT Macromolecular crowding extends the range of ionic conditions supporting high DNA polymerase reaction rates. Reactions tested were nick-translation and gap-filling by DNA polymerase I of Escherichia coli, nuclease and polymerase activities of the large fragment of that polymerase, and polymerization by the T4 DNA polymerase. For all of these reactions, high concentrations of nonspecific polymers increased enzymatic activity under otherwise inhibitory conditions resulting from relatively high ionic strength. The primary mechanism of the polymer effect seems to be to increase the binding of polymerase to DNA. We suggest that this effect on protein–DNA complexes is only one example of a general "metabolic buffering" action of crowded solutions on a variety of macromolecular interactions.

The high concentrations of macromolecules within living cells (4) may be expected to alter many cellular reactions (2, 3). We have been particularly interested in the effects of such crowding on interactions of nucleic acids with themselves or with proteins (4–7) and here extend these studies to the DNA polymerase reaction. We find macromolecular crowding causes significant increases in the binding of DNA polymerase I (Pol I) of Escherichia coli or the T4 DNA polymerase to their template primers (see ref. 8 for review of DNA polymerases and related nucleases). These increases in binding are large enough to counterbalance the otherwise strongly inhibitory effects of high ionic strength, inhibitory effects that are readily tolerated by the organism but that cause large decreases in enzyme activity when assayed in vitro. Crowding effects may thus increase the range of internal environments in which the cell works efficiently as compared to the more restricted range of environments supporting high activity of isolated, purified components.

MATERIALS AND METHODS

Enzyme Assays. All polymerase and nuclease assays (in 10 μl total volume) contained (9) 32 mM Tris-HCl buffer (pH 8.0), 3 mM MgCl₂, 1.3 mM dithiothreitol, and 2 μl of enzyme dilution using a diluent of 20% (wt/vol) glycerol, 10 mM potassium phosphate buffer (pH 7.3), 1 mM dithiothreitol, and 50 μg of bovine serum albumin per ml. Substrates for the individual assays were added as follows: polymerase activity of Pol I or of the large fragment of Pol I assayed with dATP, dCTP, dGTP, and [α-32P]dTTP; nuclease activity of large fragment of Pol I, 2 μg of [15P]d(α)[d(T)]₈ per ml. Other additions of salts or polymers were as indicated in the text. Reaction mixtures were incubated at 37°C for 15 or 30 min. Reactions were terminated by chilling and adding 0.20 ml of 10 mM Na₂P₂O₇, 0.4 mg of bovine plasma albumin per ml, and 2 mM EDTA followed by addition of 0.20 ml of 10% trichloroacetic acid. After 5 min at 0°C, the mixtures were centrifuged. For nuclease assays, aliquots of the supernatant fluids were neutralized and plated. For polymerase assays, the supernatant fluids were discarded and the pellets were washed twice by redissolving in 0.1 ml of 0.1 M NaOH and precipitation with 0.4 ml of 5% trichloroacetic acid before finally redissolving in 0.4 ml of 2 M NH₄OH and plating. 32p was measured with a low background (0.5 cpm) end-window gas-flow counter (Tracerlab, Waltham, MA). Amounts of reaction were proportional to time and enzyme concentration in each of the assay systems described above. The Km for DNA was estimated from reaction mixtures in which DNA concentration was varied by 10- to 50-fold. Km and Vmax values were obtained from reciprocal plots (10); values in replicate determinations varied <50% for either parameter.

E. coli DNA was treated with pancreatic DNase I to introduce about one single-strand break per 1000 nucleotides (estimated by exchange-labeling with T4 polynucleotide kinase on denatured samples). [32P]dAₜₙ,dT₄ₖ was prepared by an excess of the large fragment of Pol I in the assay system with dAₜₙ,dT₄ₖ described above; after Sevag extraction and ethanol precipitation, the polymer was dialyzed extensively. Other materials were obtained from the indicated sources: (dAₜₙ)₉ₕ, E. coli DNA, T4 DNA polymerase, Pol I, Ficol 70, and dextran T-70; Pharmacia; the cloned large fragment of Pol I, IBI (New Haven, CT); [α-32P]dTTP (~800 Ci/mmol); 1 Ci = 37 GBq), New England Nuclear; PEG 200, Baker or Sigma; potassium glutamate, Sigma; PEG 8000, Baker; PEG 35,000, Fluka; dithiothreitol, Calbiochem; pancreatic DNase I, Millipore; bovine plasma albumin, Miles.

RESULTS

Macromolecular Crowding Effects on E. coli Pol I. Generality of polymer effect. High concentrations of nonspecific polymers cause an extension of the range of ionic conditions that support high reaction rates. For example, under the usual uncrowded assay conditions, the nick-translation reaction of Pol I has an optimal monovalent salt concentration of ~0.1 M and is strongly inhibited at higher salt concentrations (Fig. 1, control curves) (9). In contrast, in concentrated solutions of a variety of polymers such as PEG 8000, dextran T-70, Ficol 70, or bovine plasma albumin (Fig. 1 A–D, respectively), activity is maintained even at considerably higher KCl concentrations. Monomers or small oligomers of these polymers have relatively less effect when

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compared at the same weight concentrations tested for the polymers. For example, glucose and sucrose, the monomers of dextran and Ficoll, respectively, did not cause such an effect at all (Fig. 1B and C), and PEG 200 (average $M_r$ 190–210) was considerably less effective than either PEG 8000 (average $M_r$ 7000–9000) or PEG 35,000 (Fig. 2). The requirements for relatively high concentrations and for relatively large polymers are consistent with an effect arising from macromolecular crowding (2, 3).

We have compared certain of the above results obtained with the nick-translation reaction of Pol I with similar experiments using a gap-filling assay with $dA_ndT_{10}$ as the template-primer DNA. The effects of PEG 8000 and dextran T-70 in this latter system (data not shown) were similar to those from the nick-translation assays shown in Fig. 1 A and B.

A polymer-dependent extension of the range of salt concentrations yielding high nick-translation activity also occurred for several salts other than KCl. These salts, which are important intracellularly (11–14), are potassium phosphate, potassium glutamate, and MgCl$_2$ (Fig. 3 A–C). The effects of NaCl with PEG 8000 (data not shown) were indistinguishable from those of KCl with PEG 8000 shown in Fig. 1A.

Kinetic analyses. The above results indicate that crowded solutions tend to prevent the inhibition of polymerase by higher ionic strengths. The origin of this crowding effect appears to be simply an increased binding between polymerase and its template-primer DNA.

Increased salt concentrations increase the DNA concentration required for maximal Pol I activity as illustrated in Fig. 4 (0 vs. 0.2 M KCl curves without PEG). Addition of PEG tended to prevent this salt-dependent effect (Fig. 4). A series of similar experiments indicate that addition of a polymer such as PEG 8000 or dextran T-70, which tends to prevent the salt inhibition of polymerase activity (Fig. 1 A or B), concomitantly decreases the apparent $K_m$ for DNA of Pol I (Table 1).

In comparison to the effects on the $K_m$ values, the intrinsic rates of the catalytic reaction are less affected as judged by the relatively small changes in $V_{max}$ values (Table 1). Also relatively unchanged by the presence of the polymers are the responses of polymerase to changes in concentration of its low molecular weight substrates and cofactor: the apparent $K_m$ values for deoxynucleoside triphosphates or for Mg$^{2+}$ were not significantly affected by 12% (wt/vol) PEG 8000 over the range of KCl concentrations used here (data not shown).

**Macromolecular Crowding Effects on Related Systems.** Nuclease and polymerase activities of the large fragment of E. coli Pol I. If the predominant effect of crowding is to increase the binding between enzyme and DNA, nuclease and polymerase activities of the enzyme might be similarly affected. The large fragment of Pol I (8, 15, 16), which contains only a single nuclease activity in addition to its polymerizing activity, was used to examine this question. The effects of several polymers and control materials on the polymerizing and on the $3' \rightarrow 5'$ exonuclease activities of the fragment (Fig. 5 A and B) were generally comparable to each other and to the effects on the Pol I polymerizing activity described above.

Changes in the $K_m$ for DNA for the polymerizing and nuclease activities of the fragment due to addition of PEG 8000 are summarized in Table 2. Again, the general response to changes in the KCl and polymer concentration for these activities was similar to that for the nick-translation reaction of Pol I (Table 1): the $K_m$ for DNA is increased at higher ionic strength and this salt effect tends to be reversed under crowded conditions.
**T4 DNA polymerase.** We have measured the effects of the same group of polymers and control materials tested in the preceding sections on the polymerizing activity of T4 DNA polymerase (Fig. 6). These materials caused changes that were generally similar to those that they caused on the Pol I-related activities described above. Changes in the apparent affinity constants for the polymerizing activity of the T4 DNA polymerase system (Table 3) also were consistent with the pattern of changes seen with the other activities.

**DISCUSSION**

Several groups have reported stimulation of DNA replication systems by high concentrations of polymers (17-20). The influence of the polymer was originally suggested (17) to arise from an increase in the effective concentrations of the macromolecular reactants due to excluded volume effects, and in two cases the polymer effect was found to be obliterated by raising the concentrations of purified protein components (21, 22). We have examined a number of polymerase-related systems and find a general pattern of polymer-induced changes that is consistent with this original suggestion and that has a number of implications for the roles of crowding in vivo and its use in vitro.

The activities that we have tested are the \(3' \rightarrow 5'\) exonuclease activity of the large fragment of Pol I of *E. coli* as well as the polymerization activity of Pol I, of its large fragment, or of T4 DNA polymerase. In all of these cases, the characteristic effects of crowding on enzyme activity seem to be a direct result of increased binding of enzyme to DNA. Two conditions must be satisfied to get such increased binding (2, 3). (i) To get an increase in effective concentrations, both participants in the binding reaction, polymerase and DNA, must be of sufficient molecular size to be affected by the added polymer molecules. This condition is clearly met and no doubt contributes to the large magnitude of the changes in the \(K_m\) for DNA that we see compared to those reported for systems utilizing relatively small substrates (23, 24). (ii) The complex between these participants must have an effective volume smaller than that of the sum of the individual volumes, which seems highly likely in the present system as well as in a much wider range of protein–nucleic acid or other macromolecular interactions.

The components of the binding reaction—i.e., polymerase and DNA—also participate in a variety of other interactions. Shifts in binding due to crowding will tend to alter these related interactions. Nucleotide polymerization itself can clearly be affected: if the binding reaction is rate-limiting for polymerization, then an increase in binding due to crowding will increase the rate of nucleotide incorporation. High ionic strength makes DNA binding the rate-limiting interaction in the overall polymerase reaction, as judged by the dependence of the reaction on DNA concentration—indeed, under these conditions there is an increase in the polymerization rate due to crowding. Ionic strengths in the range in which DNA

![Fig. 3](image-url)  
**Fig. 3.** Effects of PEG 8000 on the response of the nick-translation activity of *E. coli* Pol I to changes in concentrations of potassium phosphate, potassium glutamate, or MgCl\(_2\). Pol I was assayed on nicked DNA at the concentrations indicated of potassium phosphate (*A*), potassium glutamate (*B*), or MgCl\(_2\) (*C*) and in the presence of the concentrations (% wt/vol) of PEG 8000 indicated on each curve.

![Fig. 4](image-url)  
**Fig. 4.** Effects of PEG 8000 on the affinity of Pol I for DNA. Pol I was assayed on nicked DNA at the indicated concentrations of KCl at 0 or 12% (wt/vol) PEG 8000.
binding is affected by crowding commonly occur within living cells (11–14, 25–27). Further, Epstein and Schultz (26) indicate that in response to high extracellular KCl concentrations, the intracellular K⁺ concentration in E. coli can rise to >0.5 M—yet even under these conditions the rate of cell duplication is relatively unaffected. We suggest that crowding may be an important mechanism in vivo for extending the range of intracellular environments to which the organism can successfully adapt.

In addition to such effects on the total amount of binding, crowding might also influence the relative amounts of competing binding reactions. For example, the ratio of base sequence-specific binding to nonspecific binding (14, 28) might be altered, particularly if there are significant conformational differences between the protein-DNA complexes involved in the two modes. Further, crowding effects could modulate the control of protein–nucleic acid reactions by changes in ionic concentrations, as was proposed by Record and colleagues for systems such as transcription by RNA polymerase that are exquisitely sensitive to small changes in salt concentrations (14, 29).

Other reactions that will be altered by changes in the binding reaction are those resulting in denaturation of enzyme or of substrate. Since crowding may tend to force binding of a functional configuration of the enzyme to a functional configuration of the substrate, increased binding could, in principle, stabilize both components to denaturation. Preliminary experiments have demonstrated such a DNA-dependent stabilization of Pol I at elevated temperatures by PEG 8000 but not by Ficoll 70 or dextran T-70 (unpublished results). Further experiments will be necessary to assess these results.

Our results suggest a general role of macromolecular crowding in vivo. The enhanced binding between macromolecular species in living cells resulting from crowded conditions may provide a kind of "metabolic buffering," a reduction in the variation of metabolic processes that are directly or indirectly controlled by the binding reactions. This type of reduction in the variation in binding and in other related reactions in response to altered intracellular environments could be of obvious adaptive value to the organism. Further, such an effect could reduce the need to control intracellular composition, temperature, etc., and so reduce the need for

<table>
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<tr>
<th>Enzymatic activity</th>
<th>KCl, M</th>
<th>Polymer</th>
<th>Km,DNA, μg/ml</th>
<th>Vmax *</th>
</tr>
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<tr>
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<td></td>
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<td></td>
<td>0.2</td>
<td>&gt;12</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.2</td>
<td>0.5–1</td>
<td>1.2</td>
</tr>
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<td>15% PEG 8000</td>
<td>6</td>
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<td></td>
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<td>15% PEG 8000</td>
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<tr>
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</tr>
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<td></td>
<td></td>
<td>0.3</td>
<td>15% PEG 8000</td>
<td>17</td>
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*Units are pmol of thymidylate incorporated or released in 30 min, normalized to 1 unit of polymerase per ml.

![Fig. 5](image5.png)

**Fig. 5.** Effects of polymers and related materials on the KCl dependence of the polymerase (A) and nuclease (B) activities of the large fragment of Pol I. Assays were at the indicated concentrations of KCl. Where indicated, the following additions were also made: 15% (wt/vol) PEG 200, PEG 8000, or PEG 35,000; 20% (wt/vol) Ficoll 70 or sucrose; 18% (wt/vol) dextran T-70 or glucose.

![Fig. 6](image6.png)

**Fig. 6.** Effects of polymers and related materials on the KCl dependence of T4 DNA polymerase activity. Assays were at the indicated concentrations of KCl. Where indicated, the following additions were made: 15% (wt/vol) PEG 200, PEG 8000, or PEG 35,000; 30% (wt/vol) Ficoll 70 or sucrose; 27% (wt/vol) dextran T-70 or glucose.
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Table 3. Polymer and salt effects on $K_{m,DNA}$ and $V_{max}$ of the polymerase activity of T4 DNA polymerase

<table>
<thead>
<tr>
<th>KCl, M</th>
<th>Polymer</th>
<th>$K_{m,DNA}$, µg/ml</th>
<th>$V_{max}$*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>None</td>
<td>2</td>
<td>0.03</td>
</tr>
<tr>
<td>0.05</td>
<td>None</td>
<td>9</td>
<td>0.09</td>
</tr>
<tr>
<td>0.10</td>
<td>None</td>
<td>20</td>
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</tr>
<tr>
<td>0.15</td>
<td>None</td>
<td>60</td>
<td>0.01</td>
</tr>
<tr>
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<td>15% PEG 8000</td>
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<td>0.06</td>
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<td>15% PEG 8000</td>
<td>11</td>
<td>0.18</td>
</tr>
<tr>
<td>0.30</td>
<td>15% PEG 8000</td>
<td>20</td>
<td>0.03</td>
</tr>
</tbody>
</table>

*Units are pmol of thymidylate incorporated in 30 min, normalized to 1 unit of polymerase per ml.

homeostatic mechanisms that organisms have developed to control certain cellular parameters. Metabolic buffering is proposed to be a global property of the cell, altering the relative rates of large numbers of cellular reactions. It would serve to relate the degree of intracellular crowding to the functional success of the cell, providing a means by which disease states or environmental and physiological changes could affect the overall performance of the cell. In addition, changes in reaction rates due to changes in crowding provide, in principle, a simple mechanism by which the cell could sense changes in its own volume.

An inhibition by salts such as that that we observe here has been seen in many other protein–nucleic acid interactions. The origin of the salt inhibition is presumably at least in part a weakening of nonspecific electrostatic attractive forces between protein and nucleic acid causing an increase in the rate constant for dissociation (14, 30). The current assay systems were chosen to help assess the relevance of in vivo crowding effects and are poorly suited for detailed mechanistic studies. Such studies with more direct binding assays might provide interesting data on the energetics of crowding.

We have looked unsuccessfully for a change in polymerase processivity in response to crowded conditions using the large fragment of Pol I in an assay system similar to that of Fairfield et al. (31). At least under the particular set of reaction conditions tested (16), processivity was not significantly changed by high concentrations of PEG 8000 or dextran T-70. Further study of crowding effects on processivity may be indicated. Ollis and colleagues (32, 33) have suggested that the processivity of Pol I may be controlled by a flexible segment of the enzyme that can close over the DNA while the DNA is held by electrostatic interactions within a deep cleft in the protein. The types and amounts of crowding agents that might favor such a conformational change in the enzyme may be quite different from those that raise the effective concentration of the enzyme.

Finally, whatever the importance of crowding turns out to be with regard to in vivo reactions, it provides yet another parameter that can be exploited in vitro. Polymerase or other reactions carried out in crowded solutions at high salt concentrations, at elevated temperatures, or under other unusual conditions may be useful in particular contexts.

The evocative terminology and lucid writing of Allen Minton have helped us repeatedly in understanding and presenting our results on macromolecular crowding. We thank Betty Canning for expert help in the preparation of this manuscript.