Factor D is a selective single-stranded oligodeoxythymidine binding protein

M.Fry^{1,2*}, F.W.Perrino², A.Levy¹ and L.A.Loeb²

¹The Rappaport Institute for Research in the Medical Sciences and Unit of Biochemistry, Faculty of Medicine, Technion-Israel Institute of Technology, PO Box 9649, Haifa 31096, Israel and ²The Joseph Gottstein Memorial Cancer Research Laboratory, Department of Pathology SM-30, University of Washington, Seattle, WA 98195, USA

Received September 11, 1987; Revised November 20, 1987; Accepted December 1, 1987

ABSTRACT

Factor D, a protein purified from rabbit liver that selectively enhances traversal of template oligodeoxythymidine tracts by diverse DNA polymerases, was examined for the sequence specificity of its binding to DNA. Terminally [³²P]-labeled oligomers with the sequence 5'-d[AATTC(N)₁₆G]-3', N being dT, dA, dG, or dC, were interacted with purified factor D and examined for the formation of protein-DNA complexes that exhibit retarded electrophoretic mobility under nondenaturing conditions. Whereas significant binding of factor D to 5'-d[AATTC(T)₁₆G]-3' is detected, there is no discernable association between this protein and oligomers that contain 16 contiguous moieties of dG, dA, or dC. Furthermore, factor D does not form detectable complexes with the duplexes oligo(dA)·oligo(dT) or poly(dA)·poly(dT). The preferential interaction of factor D with single-stranded poly(dT) is confirmed by experiments in which the polymerase-enhancing activity of this protein is protected by poly(dT) against heat inactivation two- and four-fold more efficiently than by poly(dA) or poly(dA)·poly(dT), respectively.

INTRODUCTION

Although the role of DNA binding proteins in the replication of genomic DNA of animal cells has not been established, analysis of their modes of association with DNA and enhancement of DNA polymerase activity illuminates molecular details of DNA synthesis (see refs. 1-3 for reviews). The stimulation of DNA polymerase activity by most eucaryotic cell DNA binding proteins is not dependent on the sequence of the copied template DNA. Recently, however, we purified proteins generically designated factor D, from livers of mouse (4) and rabbit (5), that specifically enhance the traversal of template oligodeoxythymidine tracts by various DNA polymerases. Factor D from both murine and rabbit hepatocytes binds to DNA and presumably by modifying its conformation, decreases the pausing of diverse DNA polymerases at template tracts of oligodeoxythymidine, and increases the apparent processivity of DNA synthesis (5-7). In the present work, we inquired whether the sequence-specific enhancement of DNA polymerases by factor D from rabbit is correlated with a similar sequence

selectivity of its binding to DNA. It is found that factor D associates specifically with oligodeoxythymidine stretches in single-stranded DNA.

MATERIALS AND METHODS

Materials

Tritium-labeled deoxyadenosine 5'-triphosphate ($[^3\text{H}]\text{dATP}$) and $[\alpha\text{-}^{32}\text{P}]\text{deoxythymidine 5'-triphosphate}$ ($[\alpha\text{-}^{32}\text{P}]\text{dTTP}$) were products of New England Nuclear. Amersham supplied $\gamma\text{-}[^{32}\text{P}]\text{adenosine 5'-triphosphate}$ ($[^{32}\text{P}]\text{ATP}$). Unlabeled deoxynucleoside 5'-triphosphates (dNTPs), dithiothreitol (DTT), ethylene-bis (oxyethylenenitrile)-tetra acetic acid (EGTA), salmon sperm DNA, polydeoxythymidine $[(\text{dT})_{400}]$, polydeoxyadenosine $[\text{poly}(\text{dA})]$, oligodeoxyadenosine $[(\text{dA})_9]$, oligodeoxythymidine $[(\text{dT})_8]$, polydeoxyadenosine-polydeoxythymidine duplex $[\text{poly}(\text{dA})\cdot\text{poly}(\text{dT})]$, bovine serum albumin (BSA) and micrococcal nuclease were provided by Sigma Chem. Bacteriophage T4 polynucleotide kinase and *E. coli* DNA polymerase I, Klenow fragment (pol I) were products of BRL. Nuclease S1 was supplied by Boehringer. The 22-mer single strands 5'-d[AATTC(A) $_{16}$ G]-3' and 5'-d[AATTC(T) $_{16}$ G]-3' were synthesized by the phosphoramidite method (8) at the Howard Hughes Institute, University of Washington, and purified by electrophoresis on a denaturing 12% polyacrylamide gel. The oligomers 5'-d[AATTC(C) $_{16}$ G]-3' and 5'-d[AATTC(G) $_{16}$ G]-3' were similarly synthesized by Operon Technologies, San Pablo, CA, and purified by HPLC followed by gel electrophoresis. Salmon sperm DNA was heat-denatured as described (9). *E. coli* single-strand DNA binding protein (SSB) was purified as described by Bobst et al. (10).

Factor D: Factor D was purified extensively from rabbit liver as described elsewhere (5). Throughout this work, the phenyl sepharose-purified fraction of factor D was used. The enhancing factor constituted 25%-40% of the total protein in this fraction, as judged by gel electrophoresis (5).

Preparation of DNA for Protein Binding Electrophoresis Assay. Gel-purified, single-stranded oligomers of the type 5'-d[AATTC(N) $_{16}$ G]-3' were labeled with $[^{32}\text{P}]$ at their 5'-terminus in a T4 polynucleotide kinase catalyzed reaction (11). Labeled duplex oligomers were prepared in two ways: Ten-fold excess (W/W) of 5'- $[^{32}\text{P}]\text{d[AATTC(A)}_{16}\text{G}]-3'$ in 1 X SSC was heated at 65°C for 15 min with unlabeled 5'-d[AATTC(T) $_{16}$ G]-3' and the mixture was cooled slowly. Alternatively, first, ten-fold excess (W/W) of unlabeled 5'-d[AATTC(A) $_{16}$ G]-3' was similarly annealed to unlabeled 5'-d[AATTC(T) $_{16}$ G]-3'. The resulting hybrid was incubated at 26°C for 30 min in a reaction mixture for pol I-catalyzed

synthesis of DNA which contained [^{32}P]dTTP (4). Two consecutive Sephadex G-50 mini-column centrifugations were employed to remove unincorporated label from [^{32}P]dTMP that was incorporated opposite the protruding ends of the duplex (11). Unlabeled duplex polymers for use in binding competition experiments were prepared by two methods: Poly(dA)·poly(dT) duplex was digested for 30 min at 30°C with S1 nuclease to remove single-stranded ends. The trimmed hybrid was resolved from hydrolysis products by two consecutive Sephadex G-50 mini-column centrifugations (11). Measurements of absorbance at 260 m μ after this purification step, revealed that about 20% of the synthetic DNA was hydrolyzed. Alternatively, (dT)₄₀₀ was hybridized with 12-fold excess (W/W) of (dA)₉ under the annealing conditions which were described above. At this excess of (dA)₉, the single-stranded (dT)₄₀₀ is completely in hybrid with the oligomer.

Electrophoretic Mobility Retardation Assay for the Binding of Factor D to DNA.

To allow formation of complexes between proteins and DNA oligomers, factor D (30-150 ng) or *E. coli* SSB (260 ng) was incubated at 4°C for 30 min with 30 ng of labeled DNA oligomer in a final volume of 10-15 μl of 25 mM Tris-HCl buffer, pH 8.0, 1 mM EDTA, 0.5 mM DTT, 20% glycerol. To detect nucleoprotein complexes that exhibit retarded mobility on nondenaturing gels, the DNA binding mixtures were electrophoresed essentially according to Fried and Crothers (12) and Landolfi et al. (13). Gels consisted of 4% polyacrylamide-0.16% BIS in 10 mM Tris-HCl buffer pH 8.0, 1 mM EDTA (TE buffer). Following prerun of the gels in TE buffer for 10 min at 4°C under 10V X cm⁻¹, the DNA binding mixtures were loaded and electrophoresis was conducted in the cold for 2 min at 20 V X cm⁻¹ and then for 135 min at 10V X cm⁻¹. The gels were dried and autoradiographed. Although the oligomers used for the gel retardation analysis were all purified as single bands on denaturing polyacrylamide gels, 5'-d[AATTC(T)₁₆G]-3' and 5'-d[AATTC(G)₁₆G]-3' frequently exhibit two and three bands, respectively, on nondenaturing gels both in the absence or presence of added proteins, (see for instance, Figures 1 and 2). This anomalous electrophoretic behavior may be due to the formation of alternate stable conformers of each oligomer, as indicated recently by Yuhasz et al. (14).

Stabilization of Factor D Activity by Polydeoxynucleotides. In the presence of polydeoxynucleotides, the polymerase stimulatory activity of factor D exhibits increased resistance to heating or to protease digestion (see results). To quantify the DNA sequence dependence of this protective effect, the following assay was constructed: For its binding to natural or synthetic DNA, factor D (5×10^{-3} units) was incubated for 15 min at 4°C with increasing amounts of

various polydeoxynucleotides in 20 μ l reaction mixtures that contained 25 mM Tris-HCl buffer, pH 8.5, 1 mM EDTA, 0.5 mM DTT, 300 μ g/ml BSA, 20% glycerol. For each amount of added polynucleotide, duplicate samples were kept at 4°C for the determination of the initial stimulatory activity of unheated factor D, while a parallel duplicate set was heated for 18 min at 52°C and then rapidly chilled on ice. Without added polynucleotide, only 0-15% of the factor remained active after heating and this residual activity was the subtracted background for the calculation of the fraction of the activity that was protected by DNA. To determine the heat-resistant activity of factor D, the protecting DNA was first quantitatively hydrolyzed by incubating all the samples for 40 min at 37°C in the presence of 2 units/ml micrococcal nuclease, 1 mM CaCl₂. Nuclease activity was terminated by cooling the mixtures for 15 min at 4°C in the presence of 4 mM EGTA and subsequently, reaction mixtures for the measurement of enhancement of the copying of poly(dT) by pol I (6) were added in a final volume of 50 μ l. DNA synthesis was carried out for 30 min at 37°C and the extent of enhancement by factor D was determined as described (6). To calculate the fraction of activity of factor D that was protected from heat denaturation, average stimulation in the heated mixtures was divided by stimulation measured in duplicates held at 4°C in the presence of the same amount of the added polynucleotide.

RESULTS

Factor D Binds Preferentially to Oligo(dT) Tracts in Single-Strand DNA.

To examine the sequence selectivity of the binding, factor D was incubated with a set of oligomers of the general type 5'-[³²P]d[AATTC(N)₁₆G]-3' with N being dG, dA, dC, or dT. Formation of oligomer-factor D complex was monitored by the appearance of a band whose electrophoretic mobility on a nondenaturing polyacrylamide gel is slower than that of the oligomer alone. As shown in Figure 1, *E. coli* SSB binds to all four oligomers, forming SSB-DNA bands that are retarded to the same extent upon electrophoresis. Binding of SSB to the oligomers is not completely sequence-independent, and it binds more efficiently to runs of (dT)₁₆ than to (dC)₁₆, (dA)₁₆, and (dG)₁₆ (Fig. 1). In contrast to the association of SSB with all the four sequences, detectable binding of factor D is evident only with 5'-[³²P]d[AATTC(T)₁₆G]-3', whereas no significant association takes place with oligomers that include clusters of (dG)₁₆, (dA)₁₆, or (dC)₁₆ (Fig. 1). That the electrophoretically shifted band represents a complex of the labeled (dT)₁₆-containing oligomer with factor D is demonstrated

Figure 1: Electrophoretic mobility retardation of a specific complex of factor D with oligo(dT) only.

Factor D (120 ng) or *E. coli* SSB (260 ng) were incubated for 30 min at 4°C with 30 ng oligomers of the type 5'-[³²P]d[AATTC(N)₁₆G]-3', (N)₁₆ being (dG)₁₆, (dA)₁₆, (dC)₁₆, or (dT)₁₆. The binding mixtures were loaded on a nondenaturing 4% polyacrylamide gel, electrophoresed for 135 min at 4°C and the separation of factor D-DNA complex from free oligomer was detected by autoradiography as described under Methods. A retarded complex band formed by factor D only with 5'-d[AATTC(T)₁₆G]-3' is marked by an arrow.

by the increasing intensity of this band when greater amounts of factor D are incubated with the oligomer (see Fig. 3 below, and Fig. 2 in ref. 5). Since lack of evident binding of factor D to oligomers other than oligo(dT) could be due to poor sensitivity of the assay, we further established the sequence specificity of the binding of factor D to DNA by a different experimental approach. The protein was incubated with 5'-[³²P]d[AATTC(T)₁₆G]-3' in the presence of excess amounts of unlabeled oligomers that contain different (dN)₁₆ runs. As seen in Figure 2, a 6.7-fold molar excess of unlabeled 5'-d[AATTC(T)₁₆G]-3' markedly decreases the amount of labeled homologous oligomer that binds to factor D. By contrast, 20-fold molar excesses of unlabeled oligomers that have stretches of (dC)₁₆, (dA)₁₆, or (dG)₁₆, do not reduce significantly the amount of (dT)₁₆-containing oligomer that binds to factor D (Fig. 2). Furthermore, in similar experiments we find that even

Figure 2: Competition by different DNA oligomers with oligo(dT) for complex formation with factor D.

Factor D (120 ng) was interacted under standard binding conditions with 30 ng 5'-[³²P]d[AATTC(T)₁₆G]-3' (approximately 10⁴ cpm) in the presence of the indicated excesses of labeled analogous unlabeled oligomers that contained 16 contiguous residues of (dC), (dA), and (dG), or in the presence of a homologous -(dT)₁₆- oligomer or (dT)₈. Gel retardation analysis of factor D-oligo(dT) complex formation was conducted as described under Methods. An arrow marks the position of the major retarded complex band. Note that both the purified oligomer and its complex with factor D generate doublet bands under nondenaturing conditions (see Methods).

50-fold molar excess of unlabeled -(dC)₁₆- or -(dA)₁₆- oligomers do not detectably reduce the binding of factor D to the labeled -(dT)₁₆- oligomer (data not shown). Interestingly, as seen in Fig. 2, a 13.3-fold excess of unlabeled (dT)₈ does not suffice to diminish the amount of labeled (dT)₁₆-containing oligomer that is complexed with factor D. We find, however, that at ratios of (dT)₈ to -(dT)₁₆- that exceed 20-fold, the binding of factor D to -(dT)₁₆- is progressively decreased (results not presented). Hence, factor D appears to bind specifically to single-strand oligomers that include runs of oligo(dT) and the extent of this binding is positively related to the length of the (dT)_n tract.

Figure 3: Gel retardation analysis of the binding of factor D to single-strand oligo(dT) and oligo(dA) and to their annealed duplex.

The binding of factor D to the different labeled oligomers and nondenaturing gel electrophoresis were conducted under standard conditions. Annealing of excess 5'-[³²P]d[AATTC(A)₁₆G]-3' to unlabeled 5'-d[AATTC(T)₁₆G]-3' and pol I-catalyzed incorporation of [³²P]dTMP into an unlabeled duplex of these oligomers were described under Methods. An arrow marks the position of the band of factor D-bound 5'-[³²P]d[AATTC(T)₁₆G]-3'.

Lack of Binding of Factor D to Double-Stranded Oligo(dA)·Oligo(dT). Insight into the mechanism of enhancement of DNA synthesis by factor D may be gained by examining the relative preference of its binding to single- and double-stranded clusters of (dT)_n. Two methods were employed to prepare labeled hybrids of 5'-d[AATTC(A)₁₆G]-3' with 5'-d[AATTC(T)₁₆G]-3'. In the first procedure, a large excess of terminally labeled (dA)₁₆-containing oligomer was annealed to a complementary (dT)₁₆-containing oligomer (see Methods). Since factor D binds to single-stranded (dT)_n but not to (dA)_n, appearance of label in a band with retarded mobility presumably indicates binding of factor D to an oligo(dA)·oligo(dT) hybrid. In the second procedure, unlabeled complementary oligomers first were annealed and then labeled by incorporation of [³²P]dTMP opposite the single-stranded ends of duplexes (see Methods). The two differently prepared hybrids, as well as their constituent single-stranded

Figure 4: Competition by poly(dA)·poly(dT) with oligo(dT) for complex formation with factor D. Factor D was bound under standard conditions to 5'-[³²P]d[AATTC(T)₁₆G]-3' in the presence of the indicated molar excess of either the unlabeled oligomer or of poly(dA)·poly(dT) duplex whose single-stranded ends were trimmed by nuclease S1 digestion (see Methods). Nondenaturing gel electrophoresis was employed to resolve the complex of factor D with the labeled oligomer (marked by an arrow).

oligonucleotides, were tested by electrophoretic mobility retardation for the formation of complexes with factor D. As shown in Figure 3, factor D binds significantly only to single-stranded 5'-d[AATTC(T)₁₆G]-3' but not to 5'-d[AATTC(A)₁₆G]-3' or to either one of the two types of labeled hybrids. To further substantiate the preference of factor D for single-stranded oligo(dT), labeled (dT)₁₆- oligomer was interacted with the protein in the presence of progressively increased amounts of the unlabeled hybrid poly(dA)·poly(dT),

Figure 5: Protection of factor D activity against heat by various synthetic DNA polymers.

Factor D was heated for 18 min at 52°C in the presence of increasing amounts of the indicated polydeoxynucleotides. After the heat inactivation, the protecting polymers were digested by micrococcal nuclease and the residual stimulatory activity of Factor D was assayed (see Methods). Each point represents an average of duplicate determinations of protected activity.

whose single-strand ends were trimmed by S1 nuclease digestion (see Methods). As demonstrated in Figure 4, 5- and 15-fold excesses of hybrid do not reduce the level of labeled oligo(dT) in complex with factor D, and some competition becomes evident only at 25- and 50-fold excess of duplex over single-strand oligomer. In a control binding reaction, complete dilution of the bound labeled oligo(dT) takes place in the presence of 10-fold excess homologous unlabeled oligomer (Fig. 4). In a parallel experiment, binding of labeled 5'-d[AATTC(T)₁₆G]-3' to factor D was conducted in the presence of unlabeled (dT)₄₀₀ that was annealed to saturation with (dA)₉ (see Methods). Essentially identical results as in Fig. 4 were obtained with this competitor which, at a 50-fold molar excess, only partially exchanged labeled oligo(dT) in its complex with factor D (results not shown).

The Activity of Factor D is Preferentially Protected Against Heat by Single-Stranded Poly(dT). Although retardation of DNA oligomers during nondenaturing gel electrophoresis serves as a reliable indication for their association with proteins (12,13), we wished to obtain independent evidence for the specific binding of factor D to single-strand oligo(dT) tracts. We observed that, in the presence of DNA, factor D exhibits increased resistance to heat or to proteolytic digestion by trypsin. With the assumption that this augmented stability is due to association of the enhancing factor with DNA, it was

inquired whether different synthetic polynucleotides differ in their ability to stabilize factor D. Factor D was heated for 18 min at 52°C in the presence of increasing amounts of poly(dT), poly(dA), or poly(dA)·poly(dT), and micrococcal nuclease was added at an amount that completely hydrolyzed any of the protecting polydeoxynucleotides. The fraction of heat-stabilized activity of factor D was calculated by dividing average enhancing activity in heated duplicates by the activity in duplicates that contained the same amount of polydeoxynucleotide but that were held at 4°C instead of 52°C (see Methods). Figure 5 summarizes typical results that were obtained in several repeated experiments. Under the experimental conditions that were employed, maximum protection of the stimulatory activity (43%) is obtained with poly(dT), whereas poly(dA) and the duplex poly(dA)·poly(dT) are less efficient in protecting factor D and in their presence only 22% and 11%, respectively, of the factor remains active (Fig. 5). These results are interpreted as showing that by binding preferentially to poly(dT), factor D becomes more heat-stable than in the presence of poly(dA) or poly(dA)·poly(dT), to which it binds to a lower extent. Interestingly, we find that in the presence of denatured salmon sperm DNA, factor D becomes 30% heat-stable at saturation, but for maximum protection this DNA is required at a 34-fold higher concentration than poly(dT) (A. Levy and M. Fry, unpublished). This requirement for higher amounts of stabilizing heteropolymeric DNA is readily explained by the relative scarcity of oligo(dT) clusters in natural DNA. It should be cautioned, however, that quantitative comparison between results of the heat protection experiment and gel retardation analysis is invalid, since the protecting polymers/oligomer and the conditions for binding, as well as the assay systems, differ radically in the two experiments. Thus the heat protection experiment yields only qualitative confirmation of the direct measurements of complex formation.

DISCUSSION

Evidence was presented in this communication to show that factor D, a DNA binding protein that selectively enhances the copying of oligo(dT) template tracts by DNA polymerases, also binds preferentially to single-strand oligo(dT). Monitoring of the formation of factor D-DNA complex by electrophoretic mobility retardation analysis reveals that this protein binds to a single-strand DNA oligomer that contains a stretch of 16 contiguous deoxythymidine residues, but it does not associate detectably with similar oligomers that include tracts of (dG)₁₆, (dA)₁₆ or (dC)₁₆ (Fig. 1). These

results are reinforced by the demonstration that 20- to 50-fold excesses of (dC)₁₆, (dA)₁₆ or (dG)₁₆ oligomers fail to diminish the binding of (dT)₁₆ to factor D (Fig. 2). Further, we find that, whereas factor D associates with single-stranded oligo(dT), it does not bind with a similar affinity to oligo(dT)·oligo(dA), or to poly(dT)·poly(dA) duplexes. This predominant binding to single-strand oligo(dT) is indicated by an undetectable direct binding of factor D to oligo(dT)·oligo(dA) (Fig. 3), as well as by the inefficient competition by poly(dT)·poly(dA) with oligo(dT) for binding to the enhancing protein (Fig. 4). Both the sequence selectivity of the binding of factor D to DNA and its preferred association with single-stranded oligo(dT) are confirmed by an independent line of evidence that assesses the relative potency of different DNA molecules to protect the activity of factor D against heat denaturation. By presumably forming a complex with factor D, poly(dT) stabilizes at 52°C the enhancing activity two- and four-fold more efficiently than poly(dA) and poly(dT)·poly(dA) duplex, respectively (Fig. 5). The direct measurement of stabilization of factor D activity by various polymers indicates, therefore, that the complex observed by gel retardation analysis is formed between oligo(dT) and factor D, rather than with another protein contaminant of the impure enhancing factor.

Complex formation between factor D and oligo(dT) is visualized in two regions on the gel; bands appear at only about one-third of the length of the gel, and occasionally (Figs. 2,3) another band of high molecular weight material appears at the top of the gel without penetrating it. We observed that the amount of the high molecular size material increased with progressive storage time of factor D and with repeated freezing and thawing of this protein. We thus regard only the first bands as representing an authentic DNA-factor D complex, whereas the high molecular size material appears to be an aggregate of DNA with partially denatured protein.

The observed electrophoretic mobility of the complex of factor D with 5'-[AATTC(T)₁₆G]-3' suggests that the protein associates with this 22-mer at a 1:1 stoichiometry. Migration of protein-DNA complexes on nondenaturing gels has been shown to be determined primarily by the size of the protein component (15), although the protein conformation also affects the electrophoretic mobility of the complex (16). The relative molecular mass of rabbit factor D is 56-63 kDa (5), whereas the molecular weight of the bound 22-mer is only 7 kDa. Hence, the size of factor D is the major determinant of the electrophoretic mobility of its complex with the oligomer. When complexed with the cooperatively bound tetrameric 74 kDa SSB, the 22-mer DNA migrates more

slowly than when it is in complex with factor D (Figure 1). Further, an oligomer complexed with the dimeric 18 kDa bacteriophage λ Cro protein migrates faster than when it is bound to factor D (F. Perrino, unpublished). The relative electrophoretic mobility of the complex of factor D with the 22-mer suggests, therefore, a 1:1 stoichiometry of DNA-protein binding. This conclusion is reinforced by the observation that in the presence of increased amounts of factor D, the single electrophoretic band that represents the complex is intensified but no additional bands that exhibit progressively decreased mobility are discerned (Fig. 3 in this work, and Fig. 2 in ref. 5).

The DNA binding properties of factor D afford some insight into the possible mechanism of its sequence-specific enhancement of the activity of DNA polymerases. We reported recently that, by selectively increasing the affinity of several DNA polymerases for oligo(dT) tracts in DNA, factor D augments the apparent processivity of traversal of these base clusters by the polymerases (5-7). Assuming the existence of a functional link between the observed (dT)_n selective binding and polymerase enhancement by factor D, it appears that by binding directly to oligo(dT) runs in template DNA, factor D enables DNA polymerases to traverse these tracts with greater facility. It is likely that after the polymerase copies the template and (dA)·(dT) base pairs are formed, factor D, which binds poorly to duplex DNA, dissociates from the nascent double strand. Runs of template oligo(dT) constitute strong pause signals for some DNA polymerases (5,7). It is, therefore, tempting to propose that by binding directly to such stretches and presumably by modifying their structure, factor D decreases their potency as obstacles for the advancement of DNA polymerases. The quantitative relationship between binding of factor D to oligo(dT) tracts and stimulation of polymerase activity is not known. For example, it is clear that factor D binds to (dT)₁₆ more strongly than to (dT)₈ (Fig. 2). Yet factor D enhances traversal by polymerase of even (dT)₄ tract in DNA (5,7). Thus, weak or partial binding can perhaps affect polymerase activity. We also do not know at present the structural basis for either the action of oligo(dT) runs as polymerase pause signals, or for the specific permissive effect of factor D at such regions. Although bending of DNA at oligo(dA)·oligo(dT) regions is well-documented (17-19), it is unlikely that factor D associates with such bent domains, since it does not bind to duplex DNA. It appears more likely that factor D binds to DNA rendered single-stranded during the course of DNA replication. This association might, however, temporarily prevent the formation of bends in copied oligo(dT) template stretches.

ACKNOWLEDGEMENTS

We are indebted to M. Horwitz for helpful discussions and generous advice and to R. Sharf and P. Weisman-Shomer for able technical help.

This work was supported by grants from the United States-Israel Binational Science Fund, Jerusalem, Israel and from the Fund for the Promotion of Research in the Technion (M.F.) and by NIH grant AG 01751 (L.A.L.).

*To whom correspondence should be addressed

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