Isolation of a Yeast Single-Strand Deoxyribonucleic Acid Binding Protein That Specifically Stimulates Yeast DNA Polymerase I†

Stephen G. LaBonne‡ and Lawrence B. Dumas*

ABSTRACT: We sought a protein from yeast that would bind more strongly to single-stranded DNA than to duplex DNA and would stimulate the activity of the major yeast DNA polymerase, but not polymerases from other organisms. We isolated a protein that binds about 200 times more strongly to single-stranded DNA than duplex DNA and stimulates yeast DNA polymerase I activity 4–5-fold. It inhibits synthesis catalyzed by calf thymus DNA polymerase α and has little effect on T4 DNA polymerase. This yeast protein, SSB-1, has a molecular weight of approximately 40000. At apparent saturation there is one protein molecule bound per 40 nucleotides. Protein binding causes the single-stranded DNA molecule to assume a relatively extended conformation. It binds to single-stranded RNA as strongly as to DNA. SSB-1 increases the initial rate of polymerization catalyzed by yeast DNA polymerase I apparently by increasing the processivity of the enzyme. We estimate there are 7500–30000 molecules of SSB-1 per yeast cell, enough to bind at least 400–1600 nucleotides per replication fork. Thus it is present in sufficient abundance to participate in DNA replication in vivo in the manner suggested by these in vitro experiments.

Single-stranded DNA-binding proteins, or SSBs [a term suggested by Meyer et al. (1979)], may be defined as non-sequence-specific DNA-binding proteins which lack enzyme activity and bind significantly more strongly to single-stranded than to double-stranded DNA. Such proteins have been implicated in DNA replication in a variety of organisms [for a recent review, see Kowalczykowski et al. (1981)].

Genetic studies have demonstrated that certain prokaryotic cell SSBs such as the phage T4 gene 32 protein (Alberts et al., 1968) and the *Escherichia coli* SSB (Meyer et al., 1979) are required for the replication of the genomes which encode them. Proteins analogous to these SSBs found in prokaryotes have been isolated from several eukaryotic organisms and tissues, including the basidiomycete *Ustilago maydis* (Banks & Spanos, 1975), calf thymus (Herrick & Alberts, 1976a,b; Herrick et al., 1976), mouse ascites cells (Otto et al., 1977; Richter et al., 1978), and mouse myeloma cells (Planck & Wilson, 1980; Detera et al., 1981). No genetic evidence is available linking these eukaryotic cell SSBs to specific in vivo functions, but each has been shown to interact in vitro with a homologous DNA polymerase, in most cases producing a substantial increase in polymerization rate.

As part of an ongoing program to identify yeast (*Saccharomyces cerevisiae*) proteins involved in DNA synthesis, we have used a general strategy for the isolation of single-stranded DNA-binding proteins (Herrick & Alberts, 1976a) to isolate from this organism a DNA polymerase stimulating factor which we designate yeast SSB-1. The isolation of this protein, its physical properties, and its interaction with nucleic acids and with yeast DNA polymerase I are described in this report.

Experimental Procedures

Nucleic Acids. Unlabeled and ^{3}H-labeled ϕX174 DNA was prepared essentially as described by Dumas et al. (1971). M13mp9 DNA (Messing, 1981) was prepared by phenol and chloroform/isoamyl alcohol extraction of phage obtained from the supernatant of an infected culture. A synthetic primer [d(TCCCAGTCACGACGT)] homologous to M13mp vectors was annealed to the M13mp9 DNA by heating a mixture of DNA with 2 molar equiv of primer to 90 °C for 5 min and allowing the mixture to cool gradually.

Yeast cytoplasmic RNA was the generous gift of John Hill. Activation of calf thymus DNA for use as a DNA polymerase template was carried out as described by Aposhian & Kornberg (1962).

A template-primer complex of $p(dG)_{10}$ and poly(dC) (average chain length approximately 100 nucleotides) was prepared by mixing 1 part $p(dG)_{10}$ to 10 parts poly(dC), by mass.

DNA–Celluloses. Native and denatured DNA–celluloses were prepared essentially as described by Alberts & Herrick (1971); each preparation contained approximately 1 mg of DNA/mL of packed bed volume.

Enzymes. Yeast DNA polymerase I was purified as described by Chang (1977), with the following exceptions: cell lysis was accomplished by shaking with glass beads as described below, Whatman DE-32 DEAE-cellulose was substituted for DE-11, and Sephacryl S-300 Superfine was substituted for Sephadex G-200.

Phage T4 DNA polymerase (electrophoretically homogeneous) was purchased from Miles and calf thymus DNA polymerase α from P-L Biochemicals.

Assays. The standard DNA polymerase assay was carried out in a reaction volume of 100 μL containing 25 mM Tris[1] (pH 8.0), 0.1 mg/mL bovine serum albumin, 10 mM $MgSO_4$, 3 mM 2-mercaptoethanol, 50 μg/mL activated calf thymus DNA, 0.1 mM each of dATP, dCTP, and dGTP, and 0.01 mM [^{3}H]dTTP (2 Ci/mmol). We define 1 unit of DNA polymerase activity as the amount which causes 1 nmol of dTTP to be incorporated into acid-insoluble material in 30 min at 37 °C, under the above conditions. For all in vitro DNA synthesis reactions, incorporation of radioactivity into acid-insoluble material was determined by precipitation with cold 10% trichloroacetic acid containing 0.1 mg/mL calf thymus DNA, collection of the precipitates on Whatman GF/A

† From the Department of Biochemistry, Molecular Biology, and Cell Biology, Northwestern University, Evanston, Illinois 60201. *Received December 2, 1982.* This investigation was supported by U.S. Public Health Service Grant GM23443 to L.B.D. from the National Institute of General Medical Sciences. S.G.L. was supported in part by National Institutes of Health Training Grant GM07291.

‡ Present address: Department of Anatomy, Case Western Reserve University, Cleveland, OH 44116.

[1] Abbreviations: Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid; PMSF, phenylmethanesulfonyl fluoride.

0006-2960/83/0422-3214$01.50/0

glass-fiber filters, and counting in toluene containing 4 g/L diphenyloxazole using a liquid scintillation spectrometer.

Assays for deoxyribonuclease activity involved incubation of 1.5 μg of fd105 single-stranded DNA (containing approximately equal amounts of circular and full-length linear molecules) with either 7.5 μg of SSB-1 or an equivalent volume (10 μL) of SSB-1 storage buffer (see below) in the presence of 10 mM $MgSO_4$. After 2 h at 37 °C, the DNA samples were phenol extracted and subjected to electrophoresis in an alkaline 0.8% agarose gel for 20 h at 25 mA. The gel was neutralized, stained with ethidium bromide, and photographed in ultraviolet light. The photograph was traced with a Joyce-Loebl densitometer, and the ratio of circular to linear DNA peaks in the experimental and control samples was compared.

Nitrocellulose filter binding assays employed Millipore type HA filters prepared by boiling for 10 min in TEMG [20 mM Tris, pH 7.5, 1 mM EDTA, 1 mM 2-mercaptoethanol, and 10% (w/v) glycerol]. Each 50-μL binding reaction included TEMG, 0.1 mg/mL bovine serum albumin, ^{3}H-labeled φX174 DNA (10 ng, giving about 13000 cpm), SSB-1, and unlabeled φX174 DNA and/or other competing nucleic acids. After incubation for 30 min at 25 °C, each sample was diluted with 1 mL of buffer (identical with that in which the reaction was carried out) and passed through a boiled nitrocellulose filter. Each filter was washed with an additional 5 mL of buffer and dried. The amount of radioactivity bound was measured as described above. Background values obtained from reaction cocktails lacking SSB-1 (which were quite low, typically 100–200 cpm) were subtracted from experimental values.

Protein determinations were performed by the method of Lowry et al. (1951) with bovine serum albumin as the standard. All samples were dialyzed extensively against deionized H_2O before assay.

Electrophoresis. The denaturing polyacrylamide gel electrophoresis procedure of Laemmli (1970) was used for monitoring chromatographic fractions and that of Weber & Osborn (1969) for molecular weight estimation; in all cases the resolving polyacrylamide concentration was 10% (w/v).

Isoelectric focusing was performed in 7.5% (w/v) polyacrylamide slabs containing 1.8% (w/v), pH 5–7, ampholyte (Bio-Lyte 5/7), 0.2% (w/v) wide-range ampholyte (Bio-Lyte 3/10), 5% (w/v) glycerol, and 6 M urea. The catholyte (top) was 0.05 M H_2SO_4 and the anolyte 0.05 M NaOH. Focusing was carried out at 30 V/cm for 24 h. The pH gradient was measured in a 1-cm strip from the gel adjacent to the sample lanes. The strip was cut into 0.5-cm high segments; each segment was extracted for 30 min in 1 mL of deionized H_2O, and the pH of each extract was determined with a combination pH electrode.

Gels were ordinarily stained with 0.1% (w/v) Coomassie blue R-250, 25% (v/v) methanol, and 10% (v/v) acetic acid for 1–3 h at 65 °C and destained electrophoretically. For silver staining, the Bio-Rad silver stain kit was used according to the manufacturer's instructions.

Protein Molecular Weight Estimation. The molecular weight of SSB-1 under denaturing and reducing conditions was determined by the NaDodSO$_4$/polyacrylamide gel electrophoresis procedure of Weber & Osborn (1969); the standards used were bovine serum albumin (M_r 66000), ovalbumin (M_r 45000), pepsin (M_r 34700), trypsinogen (M_r 24000), β-lactoglobulin (subunit M_r 18400), and lysozyme (M_r 14300).

The molecular weight under nondenaturing conditions was determined by gel filtration through a column (0.7 × 48 cm) of Sephacryl S-200 Superfine equilibrated with 10 mM Tris, pH 7.5, and 50 mM NaCl. The calibration standards were thyroglobulin (M_r 670000), bovine IgG (M_r 158000), ovalbumin (M_r 45000), and cyanocobalamin (M_r 1350).

Purification of SSB-1. The haploid yeast strain A364A was grown at 23 °C to an approximate density of 5×10^7 cells/mL in 12 L of YPD medium [1% (w/v) yeast extract, 2% (w/v) peptone, and 2% (w/v) dextrose]. The cells were collected by centrifugation at 4 °C. The cell pellet was washed with deionized H_2O and resuspended in 100 mL of cold TEMG containing 1 M NaCl, 1 mM PMSF, and 2 μg/mL pepstatin. The cell suspension was added to 500 g of chilled, aid-washed glass beads in a 1-qt plastic jar, and the cells were broken by agitating the jar for 15 min by using a Red Devil paint shaker. The lysate was removed from the beads by filtration through four layers of gauze over strong suction.

Subsequent steps were performed at 4 °C. Intact cells, debris, and mitochondria were removed from the lysate by centrifugation for 45 min at 20000*g*. Poly(ethylene glycol) 6000 (from a stock solution in 1 M NaCl) was added to the resulting supernatant to a final concentration of 6% (w/v). After 30 min the nucleic acid containing precipitate was removed by centrifugation (20 min at 8000*g*). The poly(ethylene glycol) supernatant was diluted with 9 volumes of TEMG containing 1 mM PMSF and 2 μg/mL pepstatin and pumped through a native DNA–cellulose column (1.5 × 23 cm) and a denatured DNA–cellulose column (2.5 × 8 cm) connected in series. The coupled columns were washed with 200 mL of TEMG containing 0.1 M NaCl and 2 μg/mL pepstatin and then uncoupled, and the denatured DNA column was washed with an additional liter of the same buffer. Proteins which remained bound to the denatured DNA column were eluted with an 800-mL linear gradient of 0.1–0.6 M NaCl in TEMG containing 1 mM PMSF and 2 μg/mL pepstatin. SSB-1 was located by NaDodSO$_4$/polyacrylamide gel electrophoresis (it approximately comigrates with ovalbumin in the Laemmli system); it eluted between 0.45 and 0.55 M NaCl.

SSB-1-containing fractions were pooled and dialyzed overnight against 4 L of 10 mM potassium phosphate, pH 7.2, 1 mM 2-mercaptoethanol, 2 μg/mL pepstatin, and 10% (w/v) glycerol. The dialyzed sample was passed through a column (0.9 × 6 cm) of hydroxyapatite (Bio-Gel HTP) equilibrated with the same buffer. Bound protein was eluted with a 60-mL linear gradient of 10–300 mM potassium phosphate buffer (pH 7.2) containing 1 mM 2-mercaptoethanol, 2 μg/mL pepstatin, and 10% (w/v) glycerol. SSB-1 was again located by NaDodSO$_4$/polyacrylamide gel electrophoresis; it eluted between 40 and 60 mM phosphate, ahead of most contaminating species.

Where necessary, further purification was accomplished by rechromatography on denatured DNA–cellulose by using a small column (0.9 × 5 cm) and a 60-mL elution gradient.

Purified SSB-1 samples were concentrated to about 0.5 mg/mL by dialysis against solid Ficoll followed by dialysis against 20 mM Tris, pH 7.5, 1 mM 2-mercaptoethanol, 2 μg/mL pepstatin, and 50% (w/v) glycerol. The protein was stored at −20 °C in this buffer (SSB-1 storage buffer); its DNA-binding and DNA polymerase stimulating activities were stable indefinitely under these conditions. The continued presence of pepstatin was necessary to prevent slow cleavage to a M_r 36000 form (which appeared to retain normal activity).

Results

Purification and Physical Properties. A yeast single-stranded binding protein (SSB-1) was purified by a simple

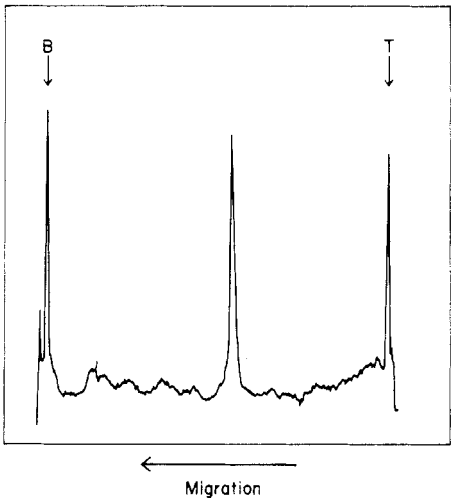

FIGURE 1: Electrophoretic profile of purified SSB-1. SSB-1 from the second DNA–cellulose purification step (10 μg) was subjected to electrophoresis under denaturing conditions in a 10% (w/v) polyacrylamide gel (Laemmli, 1970). The gel was stained by using the silver stain kit and photographed on Polaroid type 665 positive/negative film by using transmitted light. A densitometer tracing of the film is shown. The sharp spikes at the left and right ends of the tracing represent the bottom and top, respectively, of the gel. Traces of minor protein bands were visible after silver staining; only the SSB-1 band was visible after Coomassie blue staining.

Table I: Amino Acid Composition of SSB-1[a]

residue	mol/mol of protein[b]	residue	mol/mol of protein[b]
Ala	20	Lys	27
Arg	13	Phe	43
Asx	40	Ser	21
Glx	49	Thr	21
Gly	41	Trp	8
His	9	Tyr	3
Ile	30	Val	13
Leu	14		

[a] The protein sample (200 μg) was dialyzed extensively against deionized H_2O, lyophilized, and resuspended in 150 μL of 4 M methanesulfonic acid and 0.2% 3-(2-aminoethyl)indole. The sample was hydrolyzed in vacuo at 110 °C for 22 h. A 50-μL portion of the hydrolysate was neutralized and analyzed on a Durrum Model 10B automatic analyzer. [b] The values given are individually normalized to a protein molecular weight of 40 000.

procedure with DNA–cellulose and hydroxyapaptite chromatography (see Experimental Procedures). Preparations from 70 g (wet weight) of yeast cells typically yielded about 300 μg of SSB-1 after the initial DNA–cellulose selection, of which 150–200 μg could be recovered after hydroxyapatite chromatography and the second denatured DNA–cellulose step. The purity of SSB-1, after the second DNA–cellulose step, is shown by the electrophoretic profile in Figure 1.

SSB-1 has a molecular weight of 39 000 as estimated by $NaDodSO_4$/polyacrylamide gel electrophoresis and 40 000 as estimated by gel filtration under nondenaturing conditions (see Experimental Procedures for details). Thus it appears to exist as a monomer in solution. The isoelectric pH of SSB-1, as measured by isoelectric focusing in polyacrylamide gel, is 5.8. The amino acid composition is given in Table I.

Enzymatic Activities. SSB-1 preparations were found to be devoid of DNA polymerase and deoxyribonuclease (endonuclease) activity when assayed as described under Experimental Procedures.

Sedimentation Analysis of Protein–DNA Complexes. ^{3}H-Labeled φX174 DNA was incubated with varying amounts of SSB-1 and sedimented through isokinetic sucrose gradients

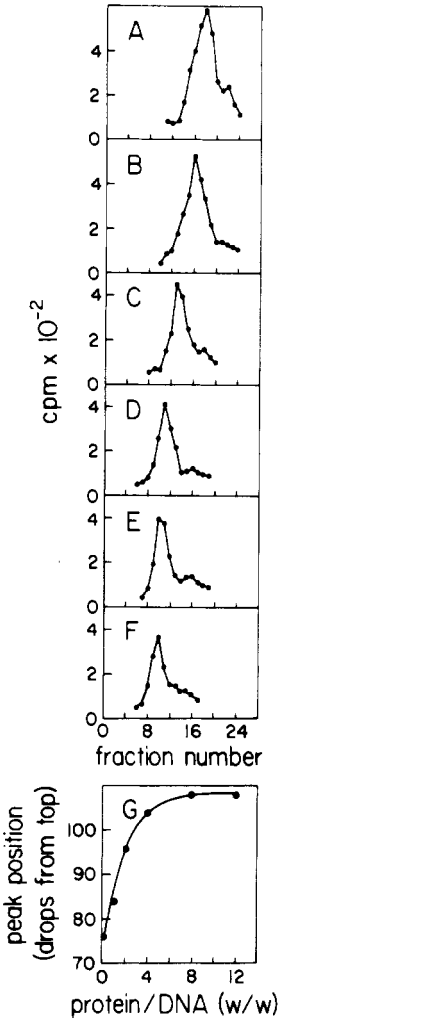

FIGURE 2: Zone sedimentation of single-stranded DNA complexed with SSB-1. Each sample (150 μL) contained 0.5 μg of ^{3}H-labeled φX174 DNA (13 000 cpm) in 20 mM Tris, pH 7.5, 1 mM EDTA, 1 mM 2-mercaptoethanol, and 50 mM NaCl (TEMN). SSB-1 was added to achieve protein to DNA ratios of 0, 1, 2, 4, 8, and 12 (samples A, B, C, D, E, and F, respectively). After incubation for 30 min at 25 °C, each sample was layered onto a preformed isokinetic sucrose gradient [also in TEMN; initial sucrose concentration 5% (w/v) prepared according to the parameters given by McCarty et al. (1974) for the Beckman SW56 rotor]. The gradients were spun 1.5 h at 55 000 rpm at 20 °C and fractionated with an automatic drop counter. The fractions were dispersed in a scintillation cocktail, and total cpm was measured. Each point in G represents the position of the most radioactive fraction in the respective gradient.

(McCarty et al., 1974); the results are summarized in Figure 2. At each protein/DNA ratio a single sedimentation band was obtained, and the sedimentation rates of the complexes increased with their protein content. No further increase in sedimentation rate occurred beyond that seen at a 10/1 protein to DNA ratio. The protein to DNA weight ratio at saturation, estimated from the extrapolation of the initial slope and plateau regions in Figure 2G to their intersection, is 3. From this we estimate one protein monomer bound per approximately 40 nucleotides at saturation.

Since in isokinetic gradients sedimentation rate is constant throughout the path length, it can be determined directly from the data in Figure 2 that the saturated protein–DNA complex sedimented 1.4 times faster than naked φX174 DNA under the conditions of this experiment. If the complex had a conformation, thus a frictional coefficient, identical with that of naked DNA, then the difference in sedimentation rates would be determined solely by the 4-fold greater mass of the complex.

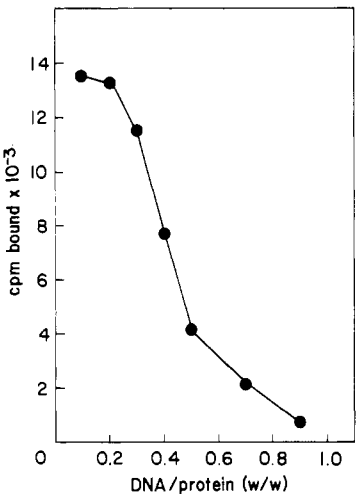

FIGURE 3: Nitrocellulose filter assay of SSB-1 binding to ϕX174 DNA. The assay was carried out as described under Experimental Procedures. Each binding reaction contained 0.5 μg of SSB-1 and 13 000 cpm of ^{3}H-labeled ϕX174 DNA. The specific activity of the latter was varied to give the indicated DNA/protein ratios.

Since protein binding to a DNA molecule increases its sedimentation coefficient in direct proportion to its increase in mass, this would mean a 4-fold increase in sedimentation rate in these isokinetic gradients upon binding of a saturating amount of SSB-1. As the actual increase was much smaller, the saturated complex presumably adopted a relatively extended conformation.

Relative Affinity of SSB-1 for Single-Stranded DNA, Double-Stranded DNA, and Single-Stranded RNA. The nitrocellulose filter binding assay described under Experimental Procedures was used to assess the affinity of SSB-1 for various nucleic acids relative to its affinity for ϕX174 single-stranded DNA. Figure 3 shows the results of an experiment in which the amount of SSB-1 was kept constant while the amount of ϕX174 DNA was varied over a 9-fold range. The sigmoidal shape of the binding curve may result from cooperative binding of SSB-1 to single-stranded DNA (see Discussion). However, a substantial portion of the binding curve approximates a straight line; within the linear region of the curve in Figure 3 doubling the DNA/protein ratio halved the retention of radioactivity. This allowed us to determine relative strength of SSB-1 binding to competing nucleic acids simply by measuring the amount of unlabeled competitor required to reduce the filter-bound radioactivity by 50%.

In the experiments depicted in Figure 4A,B, such a competition assay was carried out with double-stranded calf thymus DNA. In the low ionic strength TEMG buffer, SSB-1 bound about 40 times more weakly to calf thymus DNA than to ϕX174 DNA (Figure 4A); this ratio increased to about 200 when 0.1 M NaCl was added to the buffer (Figure 4B). This presumably more closely approximates the ionic conditions in the cell. An affinity ratio of about 40 (in TEMG) was also observed with phage f1 replicative form DNA as the double-stranded competitor (not shown). This suggests that the calf thymus DNA sample had no more single-stranded regions affecting SSB-1 binding than found in f1 duplex DNA.

As for RNA, it may be seen in Figure 4C that yeast total cytoplasmic RNA competed on an equal basis (by mass) with ϕX174 DNA. Thus, SSB-1 does not distinguish between the two species. A similar result was obtained with yeast tRNA (not shown).

Effect of SSB-1 on DNA Synthesis in Vitro. The yeast SSB-1 was added to DNA synthesis reactions including yeast DNA polymerase I, calf thymus DNA polymerase α, or

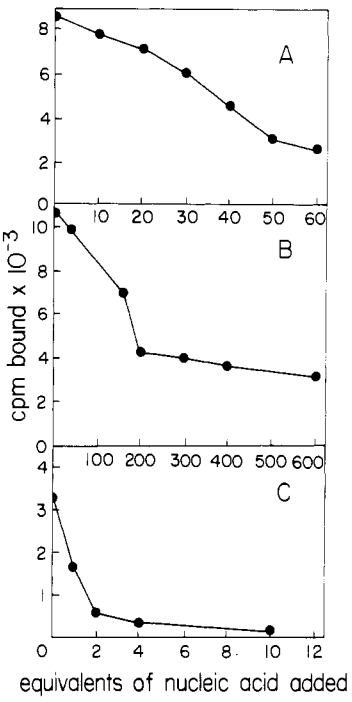

FIGURE 4: Competition of native calf thymus DNA and yeast cytoplasmic RNA with ϕX174 DNA for binding to SSB-1. Nitrocellulose binding assays were carried out as described under Experimental Procedures. (A) Each reaction contained 180 ng of SSB-1, 50 ng of ^{3}H-labeled ϕX174 DNA (13,000 cpm), and native calf thymus DNA at the indicated number of 50 ng equiv. (B) The same, except 0.1 M NaCl added to each sample. (C) The same, except only 6500 cpm of ^{3}H-labeled ϕX174 DNA added, and the competing nucleic acid was cytoplasmic RNA.

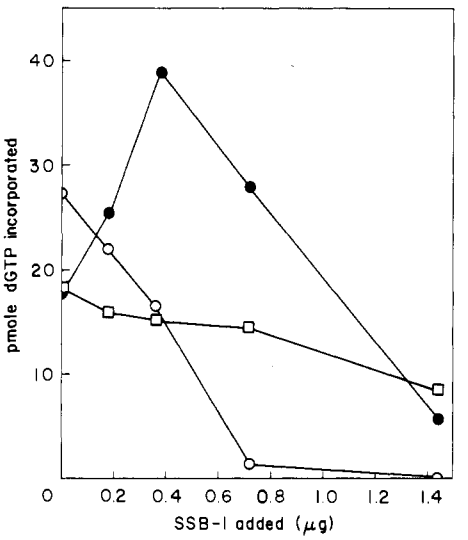

FIGURE 5: Effect of SSB-1 on three DNA polymerases. Each reaction (50 μL) included 25 mM Tris, pH 8.0, 1 mM $MgSO_4$, 3 mM 2-mercaptoethanol, 0.1 mg/mL bovine serum albumin, 0.05 mM [^{3}H]dGTP (0.4 Ci/mmol), and 2.5 μg/mL of p(dG)$_{10}$–poly(dC). The yeast DNA polymerase I (●), calf thymus DNA polymerase α (○), and T4 DNA polymerase (□) were added to a final concentration of approximately 3.4 units/mL. SSB-1 was added in the indicated amounts, while the incubation was for 30 min at 30 °C. The ratio of SSB-1 to template at maximal stimulation of the yeast polymerase was approximately 3.

bacteriophage T4 DNA polymerase. The calf and T4 polymerases were chosen for comparison because each can be stimulated by a homologous single-stranded DNA binding protein (Herrick et al., 1976; Huberman et al., 1971). The data in Figure 5 show that SSB-1 strongly inhibited calf thymus DNA polymerase α and had little effect on T4 DNA

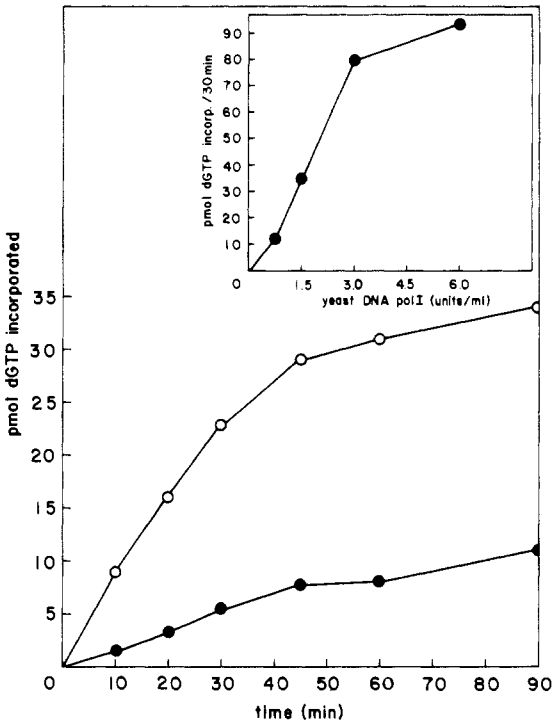

FIGURE 6: SSB-1 stimulation of yeast DNA polymerase I on p-$(dG)_{10}$-poly(dC). The reaction components were as described in Figure 5, except that DNA polymerase was added to 0.6 unit/mL. After the zero time sample was removed, 6 μL of a 0.5 mg/mL solution of SSB-1 was added to 600 μL of the reaction mixture, while 6 μL of SSB-1 storage buffer was added to a second 600-μL portion. A 100-μL portion was withdrawn from each reaction at the indicated times. Incubation was at 30 °C. (●) No SSB-1; (○) SSB-1 added. The inset shows the amounts of synthesis in 30-min reactions (100 μL) incubated at 30 °C as a function of addition of DNA polymerase I.

polymerase, under conditions where stimulation of the yeast DNA polymerase was readily observed. Thus, like other SSBs studied, stimulation seems specific to the homologous DNA polymerase. At greater than optimal levels of SSB-1, the yeast DNA polymerase was inhibited, as had been observed previously in the *U. maydis* system (Banks & Spanos, 1975). Different preparations of SSB-1 exhibited maximum stimulation at the same protein to DNA ratio.

Figure 6 shows that when SSB-1 was added at a protein to template ratio (w/w) of 2, the initial rate of polymerization on the primer-template complex p-$(dG)_{10}$-poly(dC) increased 4–5-fold. Since the poly(dC) template lacks secondary structure that might block the chain extension catalyzed by the yeast DNA polymerase, and since only approximately 0.1% of the template was copied in the first 30 min of the reaction, the increase in initial rate caused by the SSB-1 would seem to be due to facilitation of copying already available template strands rather than making more template available to the polymerase. The data in the inset to Figure 6, which show that the template can be copied much faster and to a greater extent simply by adding more DNA polymerase, are also consistent with this interpretation.

Since SSB-1 increases the initial rate of DNA synthesis on a synthetic template chain lacking secondary structure, we asked whether the SSB-1-induced increase in initial rate of DNA synthesis by the yeast polymerase might be associated with an increase in processivity (i.e., the number of nucleotides which the polymerase can add to a primer terminus before dissociating from the template-primer complex). Processivity can be indirectly measured by observing the degree to which the polymerization reaction is inhibited by the omission of one

Table II: Effect of SSB-1 on DNA Synthesis Rates in Substrate-Depleted Reactions[a]

nucleotide substrates	SSB-1/template (w/w)	pmol of dTMP incorporated in 30 min	rate (−dGTP)/rate (all)
all	0.0	4.5 ± 0.10	0.1
−dGTP	0.0	0.47 ± 0.10	
all	2.5	4.9 ± 0.26	0.08
−dGTP	2.5	0.37 ± 0.15	
all	4.0	7.8 ± 0.45	0.03
−dGTP	4.0	0.22 ± 0.10	

[a] Reaction mixtures (50 μL) included 25 mM Tris, pH 8.0, 10 mM $MgSO_4$, 3 mM 2-mercaptoethanol, 0.1 mg/mL bovine serum albumin, 2.5 μg of primed M13mp9 DNA, and 0.1 mM dATP, dCTP, and [^{3}H]dTTP (0.4 Ci/mmol). dGTP was added at 0.1 mM where indicated. Reactions were incubated at 30 °C in triplicate.

or more of the four deoxynucleoside triphosphates needed to copy natural DNA templates (Gass & Cozzarelli, 1973; Bambara et al., 1978). When primer termini are present in excess relative to DNA polymerase molecules, the processivity is inversely proportional to the ratio of the rate of DNA synthesis in the reaction where one or more substrate nucleotides are omitted to that in the complete reaction. The results of such measurements of DNA synthesis rates on primed M13mp9 DNA are shown in Table II. Note that SSB-1 increased the rate of DNA synthesis on this natural DNA template. In similar reactions on this same DNA template we observed that the stimulation by SSB-1 was even greater (up to 3-fold) when measured during the first 10 min of the reaction. The 3-fold decrease in the rate ratio seen in Table II with increasing SSB-1 concentration is consistent with an increase in the processivity of the yeast DNA polymerase.

Discussion

We have isolated from yeast a single-stranded binding protein which stimulates yeast DNA polymerase I but does not stimulate two other DNA polymerases known to respond to homologous SSBs. In this respect, and that of its nonbasic isoelectric pH, it resembles the eukaryotic SSBs mentioned in the introduction.

Another resemblance to these proteins lies in the relatively large number of molecules per cell. We were unable to quantitate SSB-1 in fractions prior to the DNA–cellulose selection and therefore must extrapolate from the amount recovered after this step. On average we obtained 300 μg of SSB-1/70 g wet weight of yeast (about 6×10^{11} cells) from the first denatured DNA–cellulose column. Assuming that the yield from the purification steps up to this point lies between 25% and 100%, we estimate that each yeast cell contains 7500–30 000 molecules of SSB-1. This could be an underestimate if considerable amounts of this protein were lost on passage through the double-stranded DNA–cellulose column. Our estimate of the number of yeast SSB-1 molecules per cell is approximately 1 order of magnitude less than those of calf thymus UP1 (Herric & Alberts, 1976a) and the *U. maydis* protein (Banks & Spanos, 1975).

The yeast SSB-1 molecule may bind cooperatively to single-stranded DNA, but the degree of cooperativity cannot be as high as that of the T4 gene 32 protein (cooperativity parameter ~2000; Kowalczykowski et al., 1981). Sedimentation showed a single band at all protein to DNA ratios tested (Figure 2). Strong cooperativity, like that of the gene 32 protein, would have been manifested by the presence of two sedimentation bands at less than saturating protein concentration, corresponding to naked DNA and the fully saturated

complex. However, the nitrocellulose filter binding data (Figure 3) showed the sigmoidal dependence of response upon protein to DNA ratio characteristic of cooperative binding. Banks & Spanos (1975) have pointed out that the shape of the filter binding curve could also be influenced by the number of protein molecules necessary to bind the complex to the filter. Thus our sedimentation and filter binding data are consistent with cooperative protein binding to single-stranded DNA (cooperativity parameter much less than that of the gene 32 protein) but do not prove that the binding is cooperative.

The sedimentation analysis indicates a protein to DNA mass ratio of 3 at saturation. From this we calculate one SSB-1 monomer bound per 40 nucleotides at saturation. This is a considerably larger DNA binding site than those estimated for the other eukaryotic cell SSBs described in the introduction, which range from 5 to 10 nucleotides.

The behavior of the yeast SSB-1 suggests a role in DNA replication. It binds to single-stranded DNA, causing it to assume a more extended conformation. We have no evidence that it removes secondary structure in template DNA, but it does stimulate the initial rate of DNA synthesis on the bound template, when catalyzed by yeast DNA polymerase I. This rate increase results at least in part from an increase in the processivity of synthesis.

The highest published estimate of the number of DNA replication origins in the yeast genome is about 380 (Newlon & Burke, 1980). Thus there are (at least) 20–80 molecules of SSB-1 per replication origin. Given two replication forks per origin, and a binding site of 40 nucleotides per SSB-1 molecule, we estimate that at least 400–1600 nucleotides per replication fork could be complexed with SSB-1. We conclude that SSB-1 is present in sufficient abundance to participate in DNA replication in the manner suggested by our in vitro experiments.

A large number of yeast mutants displaying temperature-sensitive DNA replication have recently been isolated in this laboratory (Dumas et al., 1982). It should now be possible to search these strains for one which produces a temperature-sensitive SSB-1. If such a mutant can be found, the involvement of this protein in DNA replication will be confirmed.

We note that a report has appeared (Chang et al., 1979) describing the isolation from yeast of a DNA polymerase stimulating factor with a molecular weight of 37 000. This species binds to single-stranded and double-stranded DNA and stimulates DNA polymerase α from mammalian and avian sources as well as from yeast. Thus, it is difficult to say whether this protein is related to SSB-1.

Registry No. DNA polymerase, 9012-90-2.

References

Alberts, B. M., & Herrick, G. (1971) *Methods Enzymol. 21*, 198.

Alberts, B. M., Amodio, F. J., Jenkins, M., Gutmann, E. D., & Ferris, F. L. (1968) *Cold Spring Harbor Symp. Quant. Biol. 33*, 289.

Aposhian, H. V., & Kornberg, A. (1962) *J. Biol. Chem. 237*, 519.

Bambara, R. A., Uyemura, D., & Choi, T. (1978) *J. Biol. Chem. 253*, 413.

Banks, G. R., & Spanos, A. (1975) *J. Mol. Biol. 93*, 63.

Chang, L. M. S. (1977) *J. Biol. Chem. 252*, 1873.

Chang, L. M. S., Lurie, K., & Plevani, P. (1979) *Cold Spring Harbor Symp. Quant. Biol. 43*, 587.

Detera, S. B., Becerra, S. P., Swack, J., & Wilson, S. H. (1981) *J. Biol. Chem. 256*, 6933.

Dumas, L. B., Darby, G., & Sinsheimer, R. L. (1971) *Biochim. Biophys. Acta 228*, 407.

Dumas, L. B., Lussky, J. P., MacFarland, E. J., & Shampay, J. (1982) *Mol. Gen. Genet. 187*, 42.

Gass, K. B., & Cozzarelli, N. R. (1973) *J. Biol. Chem. 248*, 7688.

Herrick, G., & Alberts, B. (1976a) *J. Biol. Chem. 251*, 2124.

Herrick, G., & Alberts, B. (1976b) *J. Biol. Chem. 251*, 2133.

Herrick, G., Delius, H., & Alberts, B. (1976) *J. Biol. Chem. 251*, 2142.

Huberman, J. A., Kornberg, A., & Alberts, B. M. (1971) *J. Mol. Biol. 62*, 39.

Kowalczykowski, S. C., Bear, D. G., & von Hippel, P. H. (1981) *Enzymes, 3rd Ed. 14*, 373.

Laemmli, U. K. (1970) *Nature (London) 227*, 680.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951) *J. Biol. Chem. 193*, 265.

McCarty, K. S., Jr., Vollmer, R. T., & McCarty, K. S. (1974) *Anal. Biochem. 61*, 165.

Messing, J. (1981) in *Third Cleveland Symposium on Macromolecules: Recombinant DNA* (Walton, A., Ed.) p 143, Elsevier, Amsterdam.

Meyer, R. R., Glassberg, J., & Kornberg, A. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 1702.

Newlon, C. S., & Burke, W. (1980) *ICN-UCLA Symp. Mol. Cell. Biol. 19*, 399.

Otto, B., Baynes, M., & Knippers, R. (1977) *Eur. J. Biochem. 73*, 17.

Planck, S. R., & Wilson, S. H. (1980) *J. Biol. Chem. 255*, 11547.

Richter, A., Knippers, R., & Otto, B. (1978) *FEBS Lett. 91*, 293.

Weber, K., & Osborn, M. (1969) *J. Biol. Chem. 244*, 4406.