Proc. Natl. Acad. Sci. USA
Vol. 80, pp. 2442–2446, May 1983
Biochemistry

Affinity purification of bacteriophage T4 proteins essential for DNA replication and genetic recombination

(helix-destabilizing protein/gene 32 protein/protein–protein interaction)

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Contributed by Bruce M. Alberts, December 30, 1982

ABSTRACT **The bacteriophage T4 helix-destabilizing protein, the product of gene 32, has been immobilized on an agarose matrix and used for affinity chromatography of lysates of T4-infected *Escherichia coli* cells. At least 10 T4-encoded early proteins and 3 or 4 host proteins are specifically retained by this gene 32 protein column. Nine of the T4 proteins have been identified as being involved in either DNA replication or genetic recombination. Notably, the T4 DNA polymerase (gene 43 protein) and two major proteins in the recombination pathway (the products of genes *uvs*X and *uvs*Y) are specifically bound. On a preparative scale, the column is useful for purification of the bound proteins.**

Gene 32 of bacteriophage T4 encodes a helix-destabilizing protein that is known to be required for the processes of T4 DNA replication, recombination, and repair (1). The purified gene 32 protein has been shown to be an essential component of the T4 *in vitro* DNA replication system that has been reconstituted from purified proteins (2, 3). The importance of gene 32 protein is probably largely due to its ability to bind tightly and cooperatively to single-stranded regions of DNA (1, 4), but it has also been shown to bind specifically to the T4 DNA polymerase (5). In addition, direct binding has been detected between gene 32 protein and the gene 61 protein, a T4 primase/helicase component (unpublished data). These results suggest that the role of gene 32 protein in various stages of bacteriophage DNA metabolism is mediated in part through direct protein–protein interactions. This suggestion receives strong support from the results of Mosig *et al.* (6), who have characterized a large number of allele-specific extragenic suppressors of various gene 32 mutants and concluded that gene 32 protein must interact with a large number of different T4 proteins inside the cell.

Many of the protein–protein interactions involved in bacteriophage DNA metabolism appear to be relatively weak ones. Moreover, the results to be described reveal that a large number of different proteins are involved. For these reasons, we have undertaken the task of cataloguing the protein–protein interactions involving gene 32 protein by protein-affinity chromatography. This approach has several advantages: (*i*) high concentrations of immobilized proteins can be used, and the resulting high concentration of binding sites on affinity columns makes even weak protein–protein interactions detectable; (*ii*) both crude lysates and purified proteins can be tested for their binding to such matrices, allowing us both to examine known proteins for their binding to gene 32 protein and to search for unknown ones; and (*iii*) the method can be used either analytically or preparatively, facilitating large-scale purification of those proteins that bind to the column.

As part of our goal of understanding the detailed structure of the T4 DNA replication apparatus, we have used protein affinity chromatography to detect interactions between various different T4 replication proteins (7). Here we describe the associations detected between gene 32 protein and the other proteins present in a T4 bacteriophage-infected cell at early times of infection, when the central genetic processes of DNA replication, recombination, transcription, and repair are underway. This approach has allowed us to identify and to purify several proteins known to be involved in genetic recombination, and it has also given us a new tool for the purification of the T4 DNA polymerase. Greenblatt (8) has described a similar approach in the study of the function of the gene *N* protein of bacteriophage λ.

METHODS

Buffers. Column buffer was 20 mM Tris·HCl, pH 8.1/1 mM Na_3EDTA/1 mM 2-mercaptoethanol/5 mM $MgCl_2$/10% (wt/vol) glycerol; it was supplemented with 0.05, 0.2, 0.6, or 2.0 M NaCl as noted. Lysis buffer consisted of column buffer supplemented with 50 mM NaCl and the protease inhibitors 10 mM benzamidine hydrochloride and 0.5 mM phenylmethylsulfonyl fluoride.

Construction of Protein Affinity Matrices. Our early attempts to perform affinity chromatography by coupling T4 replication proteins to cyanogen bromide-activated agarose proved unsuccessful due to an unacceptably high background of nonspecific binding (unpublished observations) which was presumably caused by the residual charge known to be left on matrices prepared by this process (9). After testing several alternative procedures, we chose the commercially available Affi-Gel-10 (Bio-Rad) matrix for our experiments. This material consists of beads of agarose gel to which 10-atom spacer arms have been attached through ether linkages. The other end of the spacer is activated by an attached *N*-hydroxysuccinimide group; this group can be displaced by a primary amine on a protein, leaving the protein bound by a peptide bond to an uncharged gel bead.

To prepare a 2-ml column, approximately 1.4 g of activated matrix was washed into coupling buffer (0.1 M $NaHCO_3$/0.5 M NaCl, pH 8.0) at 0°C according to the manufacturer's instructions. The washed matrix was added directly to highly purified gene 32 protein (10) which had been dialyzed into the same coupling buffer (4–16 mg of protein in 2–10 ml of solution). In addition, an albumin control column was prepared by substituting bovine serum albumin (Worthington) for the gene 32 protein. As a second control, an identical sample of active matrix was added to the coupling buffer alone. After shaking at 4°C for at least 12 hr, any remaining active groups were blocked by adding 0.5 mmol of ethanolamine (pH 8.3) to all three matrices and agitating for another 12 hr. Each matrix was then recovered by centrifugation and washed with column buffer containing 2 M NaCl. The extent of coupling was determined by

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assaying the reaction supernatant and wash fractions for protein (11). In one case, a small quantity of ^{35}S-labeled gene *32* protein (a generous gift from Junko Hosoda) was added to the unlabeled gene *32* protein to be coupled, and the bound and free radioactivities were determined after the reaction was complete. Both of these methods revealed that about 95% of the applied protein became covalently bound to the matrix. Columns were then poured in 3-ml sterile syringes and stored at 4°C. These columns have been rerun several times and show few differences in their chromatographic properties after being stored at 4°C for up to 6 months. For longer periods of storage, the protein-agarose matrices were washed with column buffer containing 50 mM NaCl/0.02% NaN_3/50% (vol/vol) glycerol and stored at −20°C.

Preparation of a Cleared Lysate Containing Radioactive T4 Proteins. A 1-liter culture of *E. coli* B was grown to a density of 5×10^8 cells per ml at 37°C in M9 minimal medium and infected with 10 T4D bacteriophage per cell. Equal aliquots of [^{35}S]methionine (0.5–1.0 mCi total; 1 Ci = 3.7×10^{10} Bq) were added at 3, 4.5, and 6 min after infection to ensure uniform labeling throughout the course of the infection. At 8 min after infection, unlabeled methionine was added to a final concentration of 20 μg/ml and the culture was chilled in an ice bath. The cells were immediately collected by centrifugation (20 min, 4,200 × *g*, 4°C), the supernatant was discarded, and the pellet was frozen in a dry ice/ethanol bath and stored at −20°C. This procedure typically resulted in the incorporation of 50% of the label added into trichloroacetic acid-precipitable material.

Lysates were prepared by suspending the frozen pellet in 8 ml of lysis buffer (see above) and sonicating four times with 20-sec blasts from the microtip of a Branson sonifier at maximum power; the temperature was maintained near 4°C. Cell debris was removed by low-speed centrifugation (15 min, 9,800 × *g*, 4°C), and the supernatant was cleared of particles that sediment at >70 S by high-speed centrifugation (150 min, 100,000 × *g* in a Spinco Ti 50 rotor). The supernatant was treated at 0°C for 30 min with a mixture of pancreatic DNase I (10 μg/ml) and micrococcal nuclease (3 μg/ml) after addition of $CaCl_2$ to 1 mM. This treated clear lysate was then applied directly to affinity columns.

Uninfected *E. coli* B cells were labeled by a single addition of [^{35}S]methionine to a culture of 4×10^8 cells per ml at 37°C in M9 minimal medium. Labeling was allowed to proceed for 30 min and then the cells were collected and a lysate was prepared as for infected cells. Infected cells labeled with ^{32}P were prepared by adding [^{32}P]phosphate (0.5 mCi) to a 1-liter culture of *E. coli* B growing in a Tris-based, low-phosphate medium at 1×10^7 cells per ml. Growth was continued at 37°C to a density of 4×10^8 cells per ml. T4 bacteriophage were then added and, after 8 min, cells were collected and a cleared lysate was prepared as described above.

RESULTS

Bacteriophage T4-Encoded Proteins Are Preferentially Retained by a Column Containing Immobilized T4 Gene *32* Protein. *E. coli* cells were infected by T4 bacteriophage and the bacteriophage-encoded proteins made at early times after infection were selectively labeled with [^{35}S]methionine. One aliquot of a radioactive cleared lysate was chromatographed on an agarose gel column containing covalently bound gene *32* protein; an identical aliquot was chromatographed on an agarose gel column containing covalently bound bovine serum albumin. As an additional control, a third aliquot of the lysate was chromatographed on an identically treated agarose gel column containing no bound protein. Each column was washed extensively with column buffer containing 50 mM NaCl and then eluted with stepwise increases in NaCl concentration in the same buffer. Much more radioactive protein was retained by the gene *32* protein column than by either the albumin or the plain agarose column (Fig. 1). Because the behaviors of the two control columns were nearly indistinguishable, each binding somewhat less than 1% of the total applied radioactivity, very few proteins in the extract bound to a protein (albumin) that we expected to have little biological relevance to T4. In contrast, the gene *32* protein column retained about 9% of the total applied radioactivity (Table 1). Inclusion of 0.5% Triton X-100 in the column buffer had little effect on the nonspecific binding of proteins (data not shown).

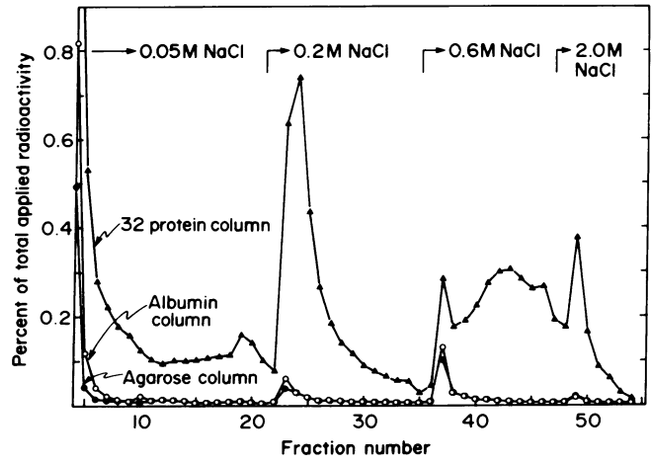

FIG. 1. Affinity matrices were prepared and equilibrated with column buffer containing 0.05 M NaCl. A cleared lysate was prepared and equal aliquots were applied to three 2-ml columns containing gene *32* protein at 8 mg/ml (0.22 mM), albumin at 12 mg/ml (0.18 mM), or no bound protein. Solutions were pumped onto the columns at 4°C at a flow rate of 3 ml/hr, and 1-ml fractions were collected. The loaded columns were washed successively with 10 column volumes of column buffer containing 0.05 M NaCl, 7 volumes of column buffer containing 0.2 M NaCl, 6 volumes of column buffer containing 0.6 M NaCl, and 4 volumes of column buffer containing 2 M NaCl. A sample from each fraction was added to scintillation fluid and the radioactivity was determined. At the end of the run, an aliquot of each column matrix was removed and treated with 4 M urea/0.2% $NaDodSO_4$ at 37°C. These samples showed that no significant amount of protein remained specifically bound after the 2 M NaCl wash; thus, all of the protein–protein interactions that can be detected on this column are salt-sensitive (data not shown).

The majority (about 90%) of the proteins present in the T4-infected cell extracts used in these experiments were the *E. coli* host cell proteins that were present before infection; these proteins were not radioactive. As will be described below, the host proteins can be detected by a two-dimensional electrophoretic analysis that distinguishes radioactive from nonradioactive proteins in the eluates. However, to facilitate a more direct com-

Table 1. Summary of eluted proteins

Matrix	% of total applied protein radioactivity eluted at each concentration of NaCl				
	0.05 M	0.2 M	0.6 M	2.0 M	Total
T4-encoded proteins					
Gene *32* protein-agarose	2.3	2.9	2.9	0.73	8.9
Albumin-agarose	0.19	0.18	0.23	0.04	0.64
Plain agarose	0.10	0.16	0.20	0.04	0.50
Host-encoded proteins					
Gene *32* protein-agarose	1.2	1.0	0.60	0.10	2.9
Plain agarose	0.30	0.23	0.14	0.04	0.70

parison of the binding of host- and T4-encoded proteins to gene 32 protein, we used a gene 32 protein column to chromatograph a radioactive extract prepared from uninfected *E. coli* cells. A relatively small proportion of the host protein bound to the gene 32 protein column, with only 2.2% of the total applied radioactivity binding differentially (Table 1). We therefore suggest that the much more extensive binding of T4 proteins to gene 32 protein reflects protein interactions of biological significance for the bacteriophage.

Polyacrylamide Gel Analysis Indicates that Many Different Proteins Are Specifically Bound to a Gene 32 Protein Column. In order to identify the specific proteins binding to the columns in the experiments shown in Fig. 1, pooled samples from each eluted peak of radioactivity were fractionated by $NaDodSO_4$/polyacrylamide gel electrophoresis, and the individual radioactive protein species were detected by autoradiography. In Fig. 2 it can be seen that the few proteins that bound to the albumin column also bound to the plain agarose column—i.e., these two control columns had essentially indistinguishable elution patterns—and therefore no T4-encoded proteins bound specifically to albumin. In contrast, at least 10 T4 early proteins bound specifically to the gene 32 protein column. Because the molecular weights of many of the T4 early proteins have been catalogued (14), several proteins could be identified directly by this one-dimensional analysis. Thus, it is evident from Fig. 2 that the gene 32 protein column quantitatively removes the T4 DNA polymerase (gene 43 protein) from the lysate, with most being subsequently released in the 0.2 M salt wash. This is a reassuring result because this DNA polymerase has been shown to interact directly with gene 32 protein by sucrose gradient centrifugation analyses (5).

A more complete description of the proteins that bound to the gene 32 protein column required the greater resolving power of two-dimensional gel electrophoresis (15). The products of known T4 genes have been identified with proteins in a standard gel pattern by analyzing the pattern of proteins synthesized in various T4 mutant infections and by observing the comigration of various known purified proteins with a protein spot in the pattern (16, 17). By reference to such a catalogue, we have been able to identify most of the proteins that bound to the gene 32 protein affinity column as the products of particular T4 genes (Fig. 3). These results are summarized in Table 2 and are also indicated by the identifications in Fig. 2.

The major specifically bound T4-encoded proteins were the products of genes 45 (DNA polymerase accessory protein), 43 (DNA polymerase), 32, and *uvs*X (a recombination pathway enzyme). We also were able to detect smaller amounts of the products of genes 46 and 47 (a nuclease that functions in genetic recombination), *dda* (DNA-dependent ATPase), *uvs*Y (a recombination protein first identified and shown to bind to 32 protein by Junko Hosada), and a T4-induced RNase H previously characterized in this laboratory (V. Chandler, personal communication), whose gene is unknown. Finally, we found significant

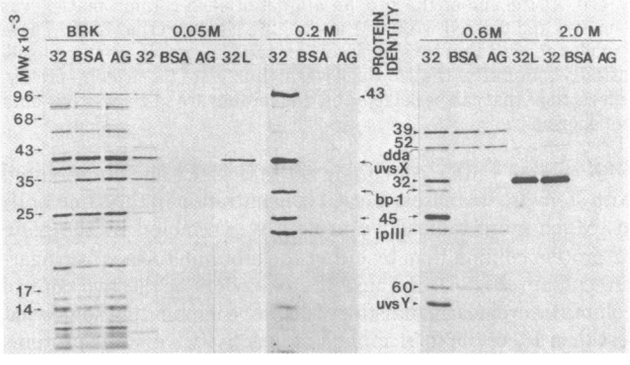

FIG. 2. Analysis of the column eluates in Fig. 1 by one-dimensional polyacrylamide gel electrophoresis. Samples from various fractions in each of the eluted peaks of radioactivity in Fig. 1 were pooled, 10 μg of insulin was added as a carrier, and the proteins were precipitated by the addition of 0.4 vol of 50% trichloroacetic acid containing deoxycholate at 2 mg/ml. Samples were held at 0°C for 30 min and the precipitate then was collected by centrifugation. The pellets were washed successively with 1 ml of acetone and 1 ml of ethyl ether, dried, and then resuspended in $NaDodSO_4$ sample buffer and fractionated by electrophoresis through a 0.75 mm thick 12.5% polyacrylamide gel composed of a 12-cm running gel overlaid with a 3-cm stacking gel (12). After electrophoresis, the gels were fixed with methanol/acetic acid/water, 5:1:5 (vol/vol), stained with Coomassie blue, impregnated with either 2,5-phenyloxazole in dimethyl sulfoxide (13) or EN^{3}HANCE (New England Nuclear), washed in water, and dried on Whatman 3 MM paper. Radioactive proteins were detected by placing the gel in contact with X-Omat R film (Kodak) at −70°C. BRK, breakthrough fractions; 32, gene 32 protein-agarose; BSA, albumin-agarose; and AG, plain agarose columns, as indicated; other elution conditions are identified by concentration of NaCl. For the 0.05 M and 0.6 M fractions, both early- and late-eluting peaks were observed from the gene 32 protein column (see Fig. 1); the samples from the latter peaks are labeled "32L." Molecular weight standards were: phosphorylase *a* (96,000), bovine serum albumin (68,000), ovalbumin (43,000), gene 32 protein (35,000), gene 45 protein (25,000), myoglobin (17,000), and lysozyme (14,000). The identification of a protein band with a specific T4 gene product is based in part on comparison with the published one-dimensional gel pattern (14), but it mostly reflects the results of the two-dimensional polyacrylamide gel analysis to be described below (see Fig. 3).

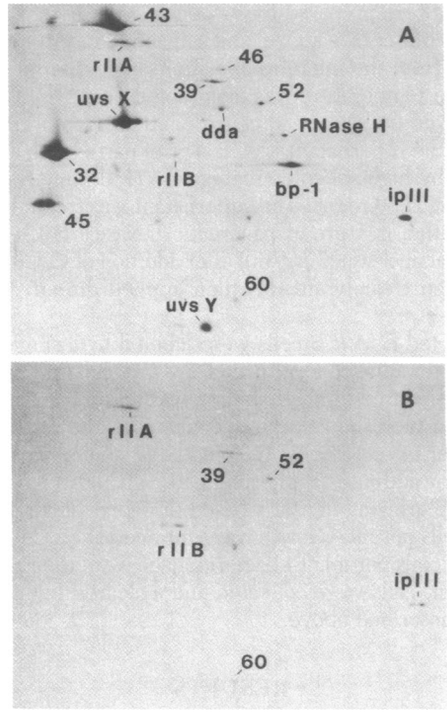

FIG. 3. Analysis of the column eluates in Fig. 1 by two-dimensional polyacrylamide gel electrophoresis. Samples of equal volume were pooled from fractions representing each eluted peak of radioactivity in Fig. 1, and the proteins were concentrated by precipitation as described for Fig. 2. The pellet was resuspended in nonequilibrium pH gradient electrophoresis sample buffer [9.5 M urea/2% (wt/vol) Nonidet P-40/1.6% Ampholines pH 5–7, 0.4% Ampholines pH 3.5–10, 5% (vol/vol) 2-mercaptoethanol/0.4% thiodiglycol], and fractionated as described by O'Farrell *et al.* (15). Proteins were detected by staining and by fluorography as in Fig. 2. (*A*) Gene 32 protein column eluates; (*B*) albumin column eluates. The indicated identifications were made by comparing the patterns obtained with a standard pattern generated as described (16, 17).

Table 2. Major protein species bound to gene *32* protein-agarose columns

Protein*	Function	Elution position, M NaCl concentration	Totally removed from lysate
T4 *32* protein	Helix-destabilizing protein	0.6	+
T4 *43* protein	T4 DNA polymerase	0.2	+
T4 *45* protein	DNA polymerase accessory protein	0.2	−
T4 *uvs*X protein	T4 recombination; recA analogue†	0.2	+
T4 *uvs*Y protein	T4 recombination	0.6	+/−
T4 *dda* protein	DNA-dependent ATPase; helicase	0.6–2.0	+
T4 *46/47* protein	T4 recombination; exonuclease	Variable	−
T4 RNase H	Removes RNA from RNA·DNA hybrids	0.2	−
BP-1	Unknown, 30,000 daltons, T4-encoded	0.2	+
BP-2	Unknown, 32,000 daltons, host-encoded	0.6	?

* For literature references to T4 gene products, see ref. 18.
† T. Minagawa, unpublished observations (cited in ref. 19); and unpublished results.

amounts of a 30,000-dalton T4 protein that has not yet been identified with any function. Note that even some of the proteins that were minor components of the eluate were removed quantitatively from the lysate—for example, the *dda* gene product (Table 2). In all cases, the identification of a protein as being encoded by T4 was based on its efficient synthesis after T4 infection, because the synthesis of *E. coli* host proteins is rapidly turned off by the infection (20).

It is also clear from Figs. 2 and 3 that the T4 type II DNA topoisomerase subunits (gene *39*, *52*, and *60* products), as well as the T4 *ipIII* protein (an internal protein bound to DNA in the phage head), were retained in greater amounts or bound more tightly on gene 32 protein than on control columns. However, because significant amounts also were bound to control columns, the meaning of this enhanced binding is unclear.

The Gene *32* Protein Column Can Be Used on a Preparative Scale. In the previous experiments, a column containing 16 mg of bound gene 32 protein in a total volume of 2 ml was used for the chromatography of extracts prepared from about 1 g of cells. A total of about 1 mg of protein was bound to the column and eluted in the subsequent salt washes. To test whether such a column is suitable for preparative-scale fractionations, we used it to remove contaminating proteins from a large-scale preparation of the T4 DNA polymerase (gene *43* protein). The starting material was DNA polymerase in fraction V from a modification of our standard preparation (21), which was about 68% pure with two major contaminating proteins (Fig. 4, lane L). When 6 mg of this protein was applied to a 2-ml gene 32 protein column, all of the DNA polymerase bound. Elution with a linear salt gradient (50–400 mM NaCl) produced a DNA polymerase >99% pure as shown by polyacrylamide gel electrophoresis (Fig. 4); the recovery of activity was quantitative. The gene 32 protein columns therefore are useful on a preparative scale as well as for analytical experiments, and they have been routinely used for the preparation of several different proteins in this laboratory.

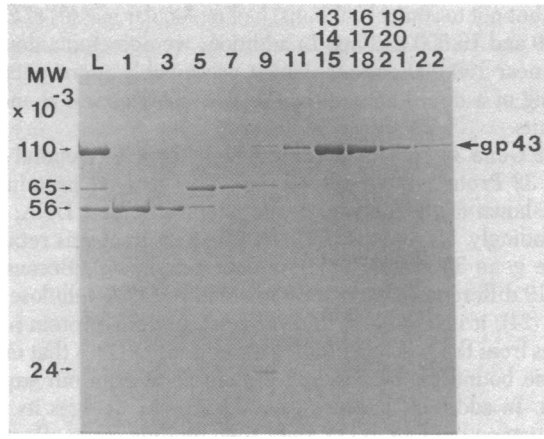

FIG. 4. Preparative-scale purification of T4 DNA polymerase on a gene *32* protein affinity column. A total of 200 g of frozen T4 (*regA*$^-$, *30*$^-$, *42*$^-$) infected *E. coli* cells were lysed and the T4 DNA polymerase was purified by a modification of the methods of Morris *et al.* (21) and Nossal and Hershfield (22) to yield fraction V. The DNA polymerase in this fraction was approximately 68% pure. A portion of this fraction was dialyzed against column buffer containing 25 mM NaCl and additional $MgCl_2$ (to 10 mM) and loaded onto a 2-ml column containing about 16 mg of covalently bound gene *32* protein as in Fig. 1. The column was washed with 1 ml of column buffer containing 0.05 M NaCl and then eluted with a 12-ml 0.05–0.4 M NaCl linear gradient in this buffer. Finally, the column was washed with 2 M NaCl. Polymerase activity eluted at about 0.25 M NaCl in this experiment; however, the elution position can vary depending on the age of the column and the concentration of immobilized gene *32* protein. Samples of selected fractions were electrophoresed as described in Fig. 2 and stained with Coomassie blue; the gel was dried between sheets of dialysis membrane. Analyzed were: 6 μl of fraction V (denoted L); 6 μl of each of fractions 1–11 as indicated; a mixture of 2.6 μl each of fractions 13, 14, and 15; a mixture of 2.6 μl each of fractions 16, 17, and 18; a mixture of 2.6 μl each of fractions 19, 20, and 21; and 10 μl of fraction 22.

Several Host-Encoded Proteins Bind Specifically to Gene 32 Protein Columns. In order to determine if infection by T4 alters the gene 32 protein binding properties of the host proteins detected in Table 1, we combined autoradiography (which detects only radioactive T4-encoded proteins) with a sensitive silver-staining technique (23) in analyzing the eluates from a gene 32 protein column. In this case, the two-dimensional polyacrylamide gel electrophoretic pattern of radioactive protein in the eluates was displayed as described for Fig. 3, and the same gel was silver-stained to detect total protein. Comparison of the autoradiograph of the gel with the total eluted proteins detected by silver-staining revealed the nonradioactive proteins that are host-encoded. By this procedure, we found that the same three or four host proteins that bound to the gene 32 protein column from an extract from uninfected cells also bound from an extract from infected cells. The major protein revealed by either method had an estimated molecular mass of about 32,000 daltons and comprised about one-fifth of the total host protein bound, being eluted essentially pure in the 0.6 M salt wash. This protein is unusual in seemingly migrating as a dimer in the $NaDodSO_4$/polyacrylamide gel unless the samples are boiled prior to electrophoresis in the $NaDodSO_4$-containing loading buffer.

In other experiments, we have discovered that this 32,000-dalton host protein binds to single-stranded DNA-cellulose, eluting between 0.05 M and 0.2 M NaCl. It has the interesting property of being specifically eluted from DNA-cellulose when 1 mM ATP is added to the eluting buffer (data not shown). This protein presumably plays a role in DNA metabolism. The other unknown host proteins that bound to the gene 32 protein col-

umn (but not to control columns) had molecular masses of about 52,000 and 16,500 daltons. In addition, we detected a doublet band near 160,000 daltons, which presumably represents the binding of a minor amount of the RNA polymerase β and β' subunits.

The Gene 32 Protein Binding Results Do Not Depend on a Gene 32 Protein–DNA Interaction. The gene 32 protein has been shown (1) to bind tightly to single-stranded DNA; correspondingly, single-stranded DNA in an extract was retained by the gene 32 protein column (data not shown). Because at least 19 different T4 early proteins bind to DNA-cellulose columns (24), it is important to distinguish protein–protein interactions from the possible binding of proteins to DNA that might become bound to the gene 32 protein column in our experiments. In addition, because gene 32 protein changes its conformation on binding to DNA (4), such binding could affect the direct interaction of gene 32 protein with other proteins. In order to eliminate nucleic acids from our experiments, extracts were treated with a mixture of nucleases prior to chromatography. However, it seemed necessary to determine directly whether a substantial amount of DNA was bound to the gene 32 protein columns. For this purpose, *E. coli* cells were labeled with [^{32}P]phosphate both prior to and during infection with T4 bacteriophage; this labeling procedure ensures that all nucleic acid in the lysate will be radioactively labeled. A cleared lysate was then prepared from these cells by our standard procedures and chromatographed on the gene 32 protein column.

The results showed that a small amount of ^{32}P-labeled material—about 0.7% of the total radioactivity remaining in the extract—bound to the gene 32 protein column under our conditions. This material appears to be a specific, protected class of small RNA molecule, and it did not contain detectable DNA (unpublished data). This RNA was present in such small quantities (about 1 nucleotide per gene 32 protein molecule) that it is unlikely to be required for the binding of any of the major proteins that were retained by this column, and it is removed early in the salt elutions. We therefore believe that any binding of nucleic acid to gene 32 protein is unrelated to the binding of the proteins listed in Table 2.

DISCUSSION

We have presented evidence that a column containing bacteriophage T4 gene 32 protein coupled to agarose beads retains at least 10 different T4 prereplicative proteins. Nine of these have been identified as being involved in T4 DNA metabolism. A few *E. coli* host proteins are also bound specifically to these columns, and at least one seems likely to be involved in DNA metabolism, although its function is unknown.

The T4 DNA polymerase was previously known to bind to gene 32 protein, and it is quantitatively retained by these gene 32 protein columns. Because gene 32 protein is relatively abundant and is easily purified in 100-mg amounts, preparative-scale columns have been constructed and are used routinely to increase the purity of our T4 DNA polymerase preparations. Another protein known to interact with gene 32 protein, the T4 gene *61* protein, has not been detected in the retained fractions. This may be due to shielding of the particular domain of gene 32 protein that binds the gene *61* protein by a nonrandom attachment of the gene 32 protein to the agarose matrix; however, alternatively it may reflect the difficulty of detecting the low amount of gene *61* protein present.

It should be noted that not all the protein associations detected here need be due to a direct interaction with gene 32 protein, because secondary complexes can also form on the immobilized gene 32 protein. For instance, purified gene *45* protein alone does not bind to these columns, whereas the same protein binds when added in crude lysates. We therefore suspect that an interaction between directly bound gene *43* protein and gene *45* protein is responsible for the binding of gene *45* protein to the column, although this has not yet been demonstrated directly.

Proteins homologous to gene 32 protein appear to be ubiquitous, are easily purified in substantial amounts (4), and are required for many facets of DNA metabolism (4, 25). Our finding that several proteins from T4 replication, recombination, and repair pathways bind to gene 32 protein columns suggests that a similar technique utilizing other helix-destabilizing proteins will prove to be of general use for studies of DNA metabolism in various organisms.

We thank Junko Hosada and Herbert Moise for sharing their data on 32 protein columns with us prior to publication. This work was supported by National Institutes of Health Grant GM 24020.

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