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Immunology

# Protein–protein interactions with the acidic COOH terminus of the single-stranded DNA-binding protein of the bacteriophage T4

(replication/T4 late transcription/eukaryotic transcriptional activation/amphipathic helix/immunodominance)

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ABSTRACT **The single-stranded DNA-binding protein of the bacteriophage T4 is encoded by gene *32*. Monoclonal antibodies were raised against intact gene *32* protein (gp32). We mapped the epitopes recognized by 12 of these monoclonal antibodies; the epitopes are all within the COOH-terminal region of gp32. As shown by others, removal of the COOH terminus of gp32 abolishes the ability of the intact protein to bind to many T4 proteins involved in replication, recombination, repair, and late transcription. These results suggest that the COOH terminus of gp32 is a protein-binding domain. The COOH terminus is attached to a DNA-binding domain that includes a zinc finger. We propose a model in which the DNA-binding and protein-binding domains are used in T4 replication, recombination, repair, and late transcription. The COOH terminus of gp32 is very acidic and may form four negatively charged amphipathic α-helices, which could fold into a four-helix bundle when associated with other proteins. At least six of the monoclonal anti-gp32 antibodies bind to the COOH terminus of gp32 and to DNA. Similarities between the COOH terminus of gp32 and DNA are explored.**

The protein encoded by gene *32* of the bacteriophage T4 is a single-stranded DNA-binding protein and participates in DNA replication, recombination and repair (1), late phage transcription (2, 3), and in the translational regulation of its own synthesis (4, 5). It has been studied extensively as a prototype for proteins that bind cooperatively and relatively nonspecifically to single-stranded nucleic acids (6, 7).

Large fragments of the gene *32*-encoded protein (gp32), lacking short peptides from either the COOH or $NH_2$ terminus (or both), can be obtained by limited proteolysis of the intact protein (6, 7). The DNA-binding properties of each large fragment differ from those of the intact protein (6, 7). Three domains that function in an integrated way to account for the specific binding parameters of the protein have been postulated (6, 7). The central domain binds to single-stranded DNA (6, 7), contains a single zinc finger (2, 8), and denatures double-stranded DNA when the $NH_2$ terminus is present (6, 7); the presence of the COOH terminus prevents this denaturation (6, 7). The $NH_2$-terminal domain participates in cooperativity (6, 7). The COOH terminus has also been implicated in various protein–protein interactions that occur during replication, recombination, repair, and late transcription (refs. 9 and 10; J. Hosoda, H. W. Moise, and W. J. Bell, personal communication); the COOH terminus is very acidic (6, 7).

We isolated mouse hybridomas producing monoclonal antibodies (mAbs) against gp32 to use as probes for each of the presumptive domains of the protein (3). Mice were immunized, and hybridomas were screened with intact gp32. mAbs generated in this way typically react with epitopes located throughout the protein (11–13). We have mapped the epitopes of the mAbs produced by 12 of these hybridomas. Interestingly, all of them react with epitopes in the COOH terminus of gp32, the very region that interacts with other T4 proteins. At least six of these antibodies, with epitopes located throughout the COOH terminus, cross-react with DNA.

## MATERIALS AND METHODS

**Materials.** Goat anti-mouse IgG and IgM alkaline phosphatase-conjugated second antibody (60527) and goat anti-mouse IgG and IgM horseradish peroxidase-conjugated second antibody (60526) were obtained from Boehringer Mannheim. Alkaline phosphatase substrates *p*-nitro blue tetrazolium chloride (170-6532) and 5-bromo-4-chloro-3-indolyl phosphate *p*-toluidine salt (170-6539) were purchased from Bio-Rad. gp32 was purified as described (3). A plasmid containing codons for amino acids 213–301 of gp32 produced the fragment called gp32(213–301). Double-stranded DNA from calf thymus (D-1501); poly(L-Asp), 15–50 kDa (P6762); poly(L-Glu), 15–50 kDa (P4761); and poly(L-Asp-L-Glu), 5–15 kDa (P1408) were from Sigma. Single-stranded DNA was prepared by immersing a solution of double-stranded DNA in a boiling water bath for 30 min followed by immediate cooling on ice. Synthetic oligonucleotides were prepared on an Applied Biosystems 380A DNA synthesizer. Monoclonal and polyclonal antibody (PC32) was produced in mouse ascites fluid (3), unless otherwise noted.

**Immunoassays.** *Noncompetitive ELISA.* This assay has been described (3). Briefly, poly(vinylchloride) plates were coated with antigen, blocked with Blotto, and incubated with antibody (3). Antibody binding was detected with goat anti-mouse IgG and IgM horseradish peroxidase-conjugated second antibody and *o*-phenylenediamine. The absorbance at 490 nm was recorded with a Flow-Titertek Multiscan.

*Competitive ELISA.* Antibody and antigen concentrations were adjusted to yield 50% maximal absorbance. Antibody was added, after competing antigen, to the antigen-coated wells. Subsequent steps were as described above.

*Immunoblot.* Separation of proteins was done on NaDodSO$_4$/15% polyacrylamide gels (2). Proteins were transferred electrophoretically from the gels to nitrocellulose sheets. Wet sheets were then cut as necessary and incubated in mouse antibody in polyclonal or monoclonal ascites fluid. Antibody binding was detected with goat anti-mouse IgG and IgM alkaline phosphatase-conjugated second antibody. The location of alkaline phosphatase activity was detected with *p*-nitro blue tetrazolium chloride and 5-bromo-4-chloro-3-indoyl phosphate *p*-toluidine salt.

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Abbreviations: gp32, protein encoded by bacteriophage T4 gene *32*; mAb, monoclonal antibody; TAG32, mAbs against T4 antigen gp32; PC32, mouse ascites fluid containing polyclonal antibody against gp32.

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## RESULTS

**COOH Terminus of gp32 Is an Immunodominant Region.** One of us has previously described the generation of mAbs against gp32 (3). These mAbs (called TAG32s for mAbs against T4 antigen gp32) were obtained by immunization and screening with intact gp32 in four separate fusions (3). We chose at random six of the TAG32s for a complete study (3). Each reacts equally well with native and denatured gp32, reacts with many fragments in partial proteolytic digests of gp32, and does *not* react with defined fragments of gp32 lacking the COOH-terminal 100 amino acids (3). The reactivity pattern of each TAG32 with a set of three fragments missing 48, 23, or ≈10 amino acids from the COOH terminus defines the section of the protein in which the epitope for each antibody is located (summarized in Fig. 1; data taken from ref. 3). Each TAG reacts with gp32(213–301), a fragment containing the COOH-terminal 89 amino acids of gp32 (Fig. 1). TAG32-2 binding to gp32(213–301) in the immunoblot assay is just barely detectable (Fig. 1). However, ELISA data establish that TAG32-2 binds to gp32(213–301) but with a lower affinity than it has for intact gp32 (data not shown). These data demonstrate that the epitopes of these mAbs are located throughout the COOH-terminal region of gp32 and define at least three separate epitopes for the six antibodies.

Often, mAbs isolated by immunizing and screening with an intact protein bind to epitopes located throughout the protein (11–13). The first six mAbs against gp32 bind to epitopes located in a single region of the protein and suggest that the COOH terminus of gp32 is an immunodominant region. To reinforce this conclusion, we tested six additional TAG32s for the ability to bind to gp32(213–301). All six bind to the fragment (Fig. 1). Thus, 12 mAbs from four separate fusions bind to epitopes in a single region of a protein. We found no mAbs that reacted with epitopes in the remaining 208 amino acids of the protein.

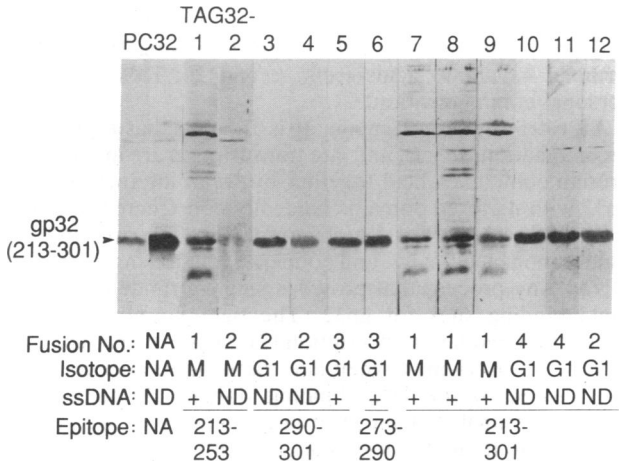


FIG. 1. Reaction of PC32 and 12 TAG32s with gp32(213–301) in immunoblot assays. The first lane is a lysate of the uninduced bacteria containing the fragment-producing plasmid pMM1. The remaining lanes are lysates of the induced bacteria. The fragment gp32(213–301) was detected with PC32 or one of 12 mAbs (TAG32s), as indicated above the blot. Below the blot the fusion (no. 1–4) from which each mAb was isolated and its isotype (G1 or M) are indicated. All TAG32s are of the κ light-chain isotype. The six mAbs that react with DNA are indicated with +. Numbers described as epitope indicate the first and last amino acid of the gp32 segment in which the antibody epitope is included (data taken from ref. 3). These numbers are known from sequencing data, except for number 290, which is estimated from the size of the fragment in protein gels (3). NA, not applicable; ND, not done. We note that gp32(213–301) runs at a $M_r$ of 18,000, which is approximately double the expected $M_r$. However, it has been demonstrated exhaustively that this fragment contains amino acid residues (213–301) of gp32 and no other gp32 sequence (L.S.G., unpublished; S. Chervitz, personal communication).

The ability of intact gp32 and gp32(213–301) to inhibit binding of PC32 to attached gp32 was compared in competitive ELISAs. The maximum percent inhibition achieved with gp32(213–301) was almost as high as that with gp32: 84% and 94%, respectively (Fig. 2), showing that almost all antibodies in PC32 bind to epitopes located in the COOH-terminal region of gp32. Thus, the epitope distribution of the mAbs reflects the epitope distribution of the entire repertoire of mouse anti-gp32 antibodies.

**TAG32s Bind to DNA.** von Hippel and coworkers (6) proposed a model in which the negatively charged COOH terminus of gp32 interacts with a positively charged DNA-binding site and is displaced from this site when the protein binds to DNA. A possible prediction of this model is that all or part of the COOH terminus resembles DNA in some way (and so can bind to the DNA-binding site) and that some antibodies that bind to the COOH terminus of gp32 might bind also to DNA. We have tested six of the TAG32s (TAG32-1, -5, -6, -7, -8, and -9) for the ability to bind to DNA. All six bind to single-stranded DNA, double-stranded DNA, and to synthetic single-stranded oligonucleotides (Fig. 3 and Table 1). This group of six includes antibodies that recognize epitopes throughout the COOH terminus (Fig. 1). We note that four of the anti-gp32/DNA antibodies cross-react, also, with a specific subset of *Escherichia coli* proteins (Fig. 1), suggesting that the elements held in common by gp32 and DNA are of even more general interest (see *Discussion*).

Both the COOH-terminal region of gp32 and DNA contain a high density of negative charge. If the binding sites of the anti gp32/anti-DNA antibodies are simply patches of positively charged amino acids, they might react with both DNA and the acidic COOH terminus via nonspecific electrostatic interactions. Such reactions might require only minimal molecular complementarity between the antibody-binding site and the epitope. If this is the case, we might expect these antibodies to bind to other molecules with a high negative-charge density. To examine this possibility, we tested TAG32-1, -5, -6, -7, -8, and -9 for the ability to bind to the negatively charged poly(amino acids) poly(L-Asp), poly(L-Glu), and poly(L-Asp-L-Glu) and to the negatively charged polysaccharide xanthan gum (14). All six bind to poly(L-Asp), but not to poly(L-Glu), poly(L-Asp-L-Glu), or xanthan gum (Fig. 3 and Table 1). The amount of poly(L-Asp) required to inhibit binding to attached DNA in competitive ELISAs is

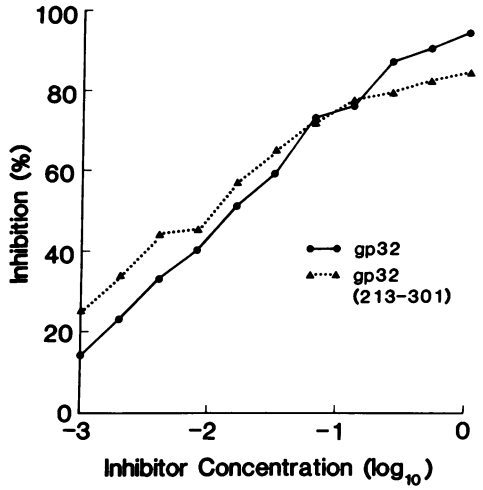


FIG. 2. Inhibition of binding of PC32 to gp32 by gp32 and gp32(213–301) was tested in a competitive ELISA. The highest concentration for each is indicated on the graph as $\log_{10}0$.

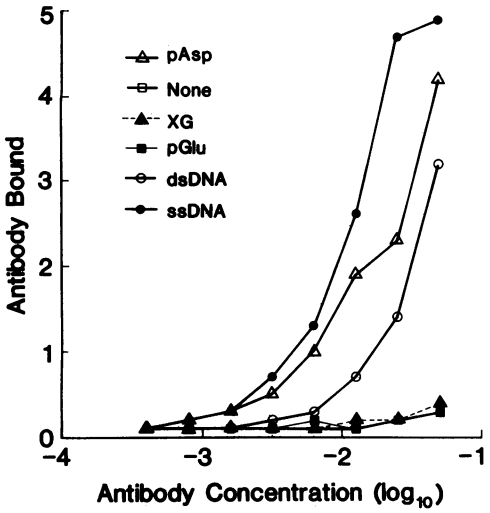


FIG. 3. TAG32-8 is in tissue-culture medium containing no fetal calf serum. Single-stranded DNA (ssDNA) at 2.5 μg per well, poly(L-Asp) (pAsp), double-stranded DNA (dsDNA), xanthan gum (XG), or poly(L-Glu) (pGlu) was attached to wells of poly(vinylchloride) plates, and binding of decreasing amounts of TAG32-8 was measured in noncompetitive ELISAs; antigen and antibody were both present in limiting amounts. Each point is the average of two or three experimental values determined in separate experiments. Antibody bound indicates absorbance units at 490 nm.

quite high (Table 1). However, we note that the antibodies bind quite well to poly(L-Asp) in the direct ELISA (Fig. 3 and data not shown). We have no explanation for this apparent difference in affinity in the two assays.

## DISCUSSION

**The Immune Response to gp32.** We isolated mAbs that react with gp32 of the bacteriophage T4 (3). Mice were immunized with intact gp32, and antibody produced by the resultant hybridomas was detected by the ability to bind to intact gp32 (3). We have mapped the epitopes of 12 of these antibodies. All 12 react with epitopes in the COOH-terminal region of the protein (Fig. 1). Almost all antibodies in PC32 react with epitopes in the COOH terminus as well (Fig. 2). Antibodies against the COOH-terminal region of gp32 dominate the immune response of the mouse to the protein.

Table 1. Inhibition of antibody binding

| | | 50% inhibition, μg per well | | | | |
|---|---|---|---|---|---|---|
| Antibody | Dilution | ssDNA | dsDNA | oligo | gp32 | pAsp |
| TAG32-1 | 1:430 | 0.009 | 0.71 | 0.13 | 0.10 | 18.0 |
| TAG32-5 | 1:100 | 0.007 | 0.17 | 0.60 | ND | 15.0 |
| TAG32-6 | 1:50 | 0.013 | 1.66 | 0.15 | 0.6 | NA |
| TAG32-7 | 1:320 | 0.007 | 0.30 | 0.15 | 0.13 | 17.0 |
| TAG32-8 | 1:530 | 0.006 | 0.26 | 0.33 | 0.10 | 27.5 |
| TAG32-9 | 1:300 | 0.008 | 0.32 | 0.27 | 0.11 | 16.5 |

Inhibition of binding of six TAG32s to 2.5 μg of attached single-stranded DNA (ssDNA) by ssDNA, double-stranded DNA (dsDNA), a synthetic 27-nucleotide oligonucleotide (oligo), gp32, poly(L-Asp) (pAsp), poly(L-Glu) (pGlu), poly(L-Asp-L-Glu) (pAsp–Glu), and xanthan gum was measured in competitive ELISAs. One hundred percent inhibition of binding to 2.5 μg of attached ssDNA was achieved with competing ssDNA, dsDNA, gp32, and the oligonucleotide. A maximum of ≈75% inhibition was achieved with 120 μg of pAsp for all TAG32s, except TAG32-6, for which 46% inhibition was achieved with 120 μg of pAsp. The amount of ssDNA, dsDNA, oligonucleotide, gp32, and pAsp required to inhibit binding to the attached DNA by 50% is given. For pGlu and pAsp–Glu, no inhibition was seen with up to 120 μg per well; for xanthan gum no inhibition was seen with up to 20 μg per well. ND, not done; NA, 50% inhibition was not achieved. All mAbs are in tissue culture medium containing no fetal calf serum.

The immune system is complex, and numerous mechanisms could be invoked to explain the immunodominance of this region (13). However, it is striking that the very region of gp32 that dominates the immune response probably binds to no fewer than nine T4 proteins involved in replication, recombination, repair, and late transcription (see next section) and participates in an intramolecular interaction in the unbound protein. We are driven by this confluence of very different experimental data to consider an idea: the COOH terminus of gp32 has a structure that is innately fit for protein–protein interaction, and the immune system recognizes and responds to this structure.

**Interaction of T4 Proteins with the COOH Terminus of gp32.** gp32 binds to at least nine T4 proteins known to be involved in T4 replication, recombination, repair, and late transcription, one to three unidentified T4 proteins, and several host proteins: these are gp32 itself (15, 16), gp61 (an RNA primase subunit) (10, 16, 17), gp43 (the DNA polymerase) (9, 16), gp45 (a protein required both for DNA replication and T4 late transcription) (16), gp46 and gp47 (subunits of a nuclease required for recombination); and dda (an ATP-dependent helicase) (16). Hosoda *et al.* (personal communication) have also reported on all of the preceding seven proteins and, in addition, found that three unidentified T4 proteins, of 19–23 kDa, 20–22 kDa, and 30 kDa, bound to a gp32 affinity column and that gp44 and gp62, the DNA polymerase accessory proteins, bound to the gp32 column under some conditions but not others. Formosa *et al.* (16) found one unidentified T4 protein of 30 kDa and a second T4 protein of 44 kDa, which has been tentatively identified as RNase H, a protein involved in primer removal from Okazaki fragments (19); three unknown *E. coli* proteins, of 16.5 kDa, 33 kDa, and 52 kDa, and two additional host proteins, tentatively identified as the RNA polymerase β and β′ subunits, also bind to a gp32 affinity column (16). Of all proteins that bind to intact gp32, only one, gp32 itself, binds to gp32 missing the COOH-terminal 48 amino acids (refs. 9 and 10; Hosoda *et al.*, personal communication).

**A Protein-Binding Domain.** It is thought that replication, recombination, repair, and late transcription are mediated by protein complexes held together by weak interactions (20). gp32 with its three domains is ideally constructed to participate in the assembly of such complexes. The protein binds tightly, nonspecifically, and cooperatively to single-stranded DNA. Any process that provides single-stranded DNA will yield binding sites for gp32. The tight coupling between replication and late transcription (21) suggests that gp32 will also be in the vicinity when late transcription occurs.

Attached to the DNA-binding domain of gp32 is the COOH-terminal domain. The large number of T4 proteins that bind to it and the predominance of antibodies against it in the immune response suggest that this region has some quality that defines it as a *protein*-binding domain. This protein-binding domain, tethered to the DNA via the DNA-binding domain, could participate in the assembly of multienzyme complexes. In the model depicted in Fig. 4, the COOH terminus is shown interacting with different subsets of proteins during recombination, repair, late transcription, and the various phases of replication. Roles for at least eight T4 proteins (other than gp32) in replication have been defined by studies with the T4 *in vitro* replication system (19, 20, 22). At least six, and possibly all eight, of these proteins bind to the protein-binding domain of gp32.

T4 late transcription has proved more refractory to analysis than replication (21). The products of T4 genes *33* and *55* bind to the *E. coli* RNA polymerase core enzyme (21). gp44, gp62, and gp45 are the T4 DNA polymerase accessory proteins; they form a complex that is required for DNA replication (19,

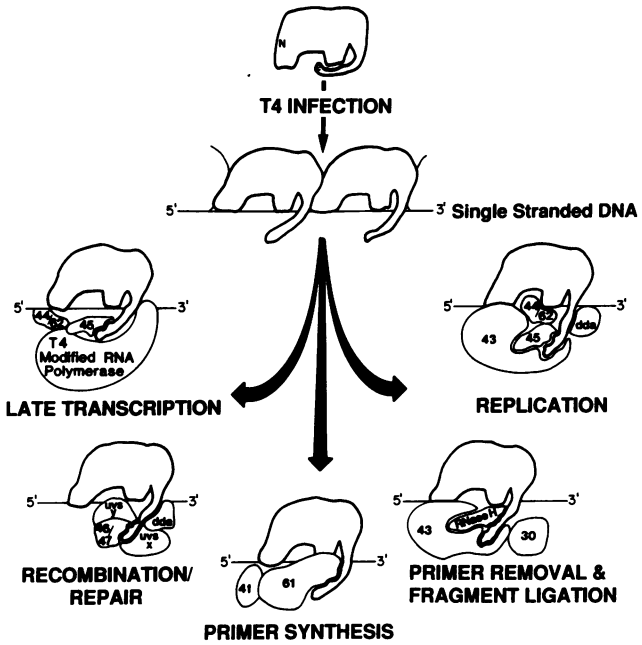


FIG. 4. Model for protein–protein interactions with the COOH terminus of gp32 during T4 infection. The COOH terminus of a free protein monomer is involved in an intramolecular interaction with the positively charged DNA-binding site. When the protein binds to single-stranded DNA, the COOH terminus is displaced from this site and is free to interact with other proteins. During T4 infection, gp32 binds to transiently single-stranded regions of DNA, freeing the COOH terminus for other interactions. The COOH terminus is shown interacting with different subsets of proteins during replication, recombination, repair, and late transcription. No attempt has been made to draw the individual molecules to scale. gp32 is depicted as large to accommodate binding to all molecules in each complex. In fact, several molecules of gp32 could be involved in each complex, each interacting with one or a subset of the other molecules. dda, ATP-dependent helicase; uvsx and uvsy, enzymes required for recombination; numbers represent additional proteins.

20). This complex stimulates transcription *in vitro* by the gp33/gp55/RNA polymerase complex from T4 late promoters on nicked double-stranded plasmid DNA (23, 24). The nick can be located many base pairs upstream or downstream from the mRNA start site but must be on the nontranscribed strand (23, 24). In this *in vitro* system, gp32 enhanced transcription by 2-fold and counteracted inhibition by the T4 DNA polymerase (23). The *in vitro* system differs in critical ways from late transcription *in vivo*: most notably, a nick attracts gp44/gp62–gp45 to the vicinity of the late promoter. Geiduschek and colleagues (23) suggest that this nick substitutes for the replication fork *in vivo* and that the replication fork might recruit other replication proteins, such as gp32, for transcriptional activation; they think of the replication fork as a "mobile enhancer" (23). In the model shown in Fig. 4 the gp32 protein-binding domain participates in a transcription complex containing the T4-modified RNA polymerase and the gp44/gp62–gp45 complex.

Two elements, a sequence-specific DNA-binding domain and a separate short acidic region that is not required for DNA binding but contacts other components of the transcription machinery, are central to general models that explain the mode of action of eukaryotic transcriptional-activation proteins (25, 26). These acidic regions show no sequence homology to each other or to *E. coli* sequences that can activate transcription in yeast (25, 26). We have discussed (2, 3) the possibility that the gp32 DNA-binding domain binds specifically to T4 late promoters, via a "eukaryotic" DNA-binding motif, the zinc finger. In this paper, we have noted that gp32 contains a separate region not required for binding to DNA but that participates in DNA metabolism by interacting with other proteins in complexes. This region of gp32 is extremely acidic; the net negative charge is −10 over 50 amino acid residues (6, 7). The model for the action of eukaryotic transcriptional-activation proteins is comparable to the model for gp32 activation of T4 late transcription that arises from the more general model of gp32 structure and function depicted in Fig. 4.

**The Protein-Binding Domain of gp32 Resembles DNA.** The model represented in Fig. 4 requires that a relatively short segment of a single protein binds to a large but specific set of proteins that have no obvious sequence or structure in common. The large number of proteins that interact with double-stranded DNA provides a metaphor for the multiple-binding specificities of the gp32 protein-binding domain. In the control regions of genes, several proteins can bind to a short DNA region, and different subsets of proteins may bind to overlapping segments at different times. Double-stranded DNA has a negatively charged helical structure.

Many protein segments known to activate transcription in yeast can theoretically form negatively charged amphipathic α-helices. A synthetic sequence, designed to encode a four-turn amphipathic helix, was able to activate transcription in yeast (25, 26). Helical-wheel representations of the amino acid sequence of the COOH-terminal region of gp32 reveal four segments that could form amphipathic helices: amino acids 225–243 (I), 250–263 (II), 266–278 (III), and 291–301 (IV). Three of these helices are resident within the "canonical" COOH-terminal domain of the protein, residues 253–301, as defined by partial proteolysis (6, 7); the fourth is in the sequence immediately adjacent to this region. The four-helix bundle is a common feature in known tertiary structures of proteins (27), so that the prediction of a fourth helix justifies extension of the COOH-terminal domain to include residues 225–252. The only residues that remain outside of the four potential helices are the extraordinary sequence $Ser_4$-Gly-$Ser_4$ and a stretch of six uninterrupted hydrophobic residues. Fig. 5 depicts these elements as part of a four-helix bundle. Like DNA, this four-helix bundle has a negatively charged structure.

At least six mAbs recognize the protein-binding domain of gp32 *and* DNA; that is, the binding sites of these mAbs exhibit molecular complementarity to the COOH terminus of gp32 and to DNA. Some resemblance between the two must exist on the molecular level. This resemblance may be accounted for by the high density of negative charge that the two molecules have in common. However, the mAbs do not react with all negatively charged molecules (Table 1), suggesting that negative charge is not in itself sufficient to account for the cross-reactivity. The likelihood that the structure of the COOH terminus of gp32 has elements in common with the structure of DNA, the intramolecular interaction between the COOH terminus and the DNA-binding site in unbound gp32, and the similar protein-binding functions of the two structures lead us to the idea that the protein-binding domain of gp32 *actually resembles* and perhaps evolved to *mimic* DNA. Specifically, we argue that *some* structure of the gp32 COOH terminus (perhaps even the four-helix bundle of Fig. 5) places the negatively charged Asp and Glu residues into the nucleic acid-binding site of gp32 so as to make ionic contacts with the amino acid residues used to contact phosphates in the nucleic acid substrate. The different chemistries of phosphorus and carbon are not likely to be a problem (28). Similarly, the mAbs that recognize both DNA and the COOH terminus must have an epitope-recognition site that complements either polyanion. We wonder, of course, whether the anti-DNA antibodies of the autoimmune disease, systemic lupus erythematosus, are elic-

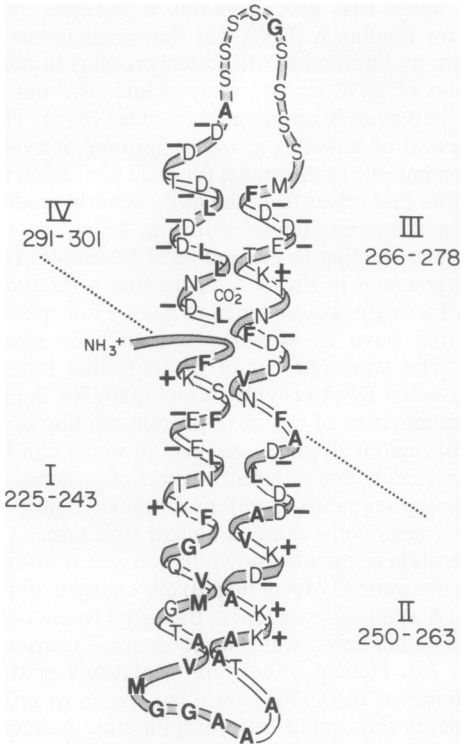


FIG. 5. Hypothetical structure for the COOH terminus of gp32. The polypeptide backbone of amino acid residues of gp32 is shown schematically. α-Helices are depicted as helical ribbons. This region of gp32 consists of four amphipathic helices labeled I, II, III, and IV, the sequence $Ser_4$-Gly-$Ser_4$ (top loop), and a stretch of six uninterrupted hydrophobic residues (bottom loop). The four helices are shown as two pairs, each with its hydrophobic face turned inward toward its partner. In the three-dimensional structure, the two pairs might come together (fold on the dotted line), forming a four-helix bundle. Amino acids are indicated using the one-letter code. Boldface letters indicate hydrophobic residues. Positive (+) and negative (−) charges are indicated.

ited by proteins with sequence/structure similarities to the COOH terminus of gp32 (29).

The amount of gp32(213–301) required to achieve 50% inhibition of antibody binding to attached gp32 in competitive ELISAs varies for each TAG32; however, this amount is always significantly greater than the amount of gp32 required (data not shown). This result suggests that the structure of this peptide differs in isolation and in the intact protein. Preliminary results of CD and NMR analysis of purified gp32(213–301) suggest a random-coil or flexible configuration for the isolated peptide (S. Chervitz, A. Pardi, and D. Barrick, personal communication). The structure of this region in the intact protein is unknown.

The gp32 protein-binding domain may be structureless in isolation because the density of negative charge in the structure may make its formation energetically unfavorable. In the intact protein, this domain interacts with a positively charged DNA-binding site, and during replication, recombination, repair, and late transcription it interacts with presumably positively charged regions of other proteins. A folded structure may become energetically favorable when the high negative-charge density is neutralized by contact with positively charged regions of the core or other proteins. Such a change from a flexible to a rigid structure upon ligand binding is an accepted class of allosteric interaction and occurs, for example, when RNA binds to the coat protein of the tobacco mosaic virus (30). The disordered region of the free coat protein forms a positively charged amphipathic helix when bound to negatively charged RNA (30).

The binding of single-stranded DNA to gp32 induces a structural change in which the relative orientation of the core and the COOH terminus is altered (6, 7). It is possible that when the COOH terminus is displaced from the DNA-binding site, a second structural change occurs: the structure of the COOH terminus is altered from an ordered, rigid structure, as shown in Fig. 5, to a flexible, disordered state. Upon binding of a T4 protein or proteins to the displaced COOH terminus, the ordered structure might reform, and its folding could contribute to the formation of the putative complexes involved in T4 DNA metabolism.

The acidic amphipathic helix, composed of nucleotides or amino acids, may be a broadly useful structural motif for achieving multiple protein interactions. It may ultimately be perceived as a structural paradigm for systems in which multiple binding specificities are required of a single surface.

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