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Functional Role of the N-Terminal Domain of Bacteriophage T4 Gene Product 11

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Abstract—Bacteriophage T4 late gene product 11 (gp11), the three-dimensional structure of which has been solved by us to 2.0 Å resolution, is a part of the virus' baseplate. The gp11 polypeptide chain consists of 219 amino acid residues and the functionally active protein is a three-domain homotrimer. In this work, we have studied the role of gp11 N-terminal domain in the formation of a functionally active trimer. Deletion variants of gp11 and monoclonal antibodies recognizing the native conformation of gp11 trimer have been selected. Long deletions up to a complete removal of the N-terminal domain, containing 64 residues, do not affect the gp11 trimerization, but considerably change the protein structure and lead to the loss of its ability to incorporate into the baseplate. However, the deletion of the first 17 N-terminal residues results in functionally active protein that can complete the 11^--defective phage particles in *in vitro* complementation assay. This region of the polypeptide chain is probably essential for gp11–gp10 stable complex formation at the early stages of phage baseplate assembly *in vivo*. A study of the gp10 deletion variants suggests that the central domain of gp10 trimer is responsible for the interaction with gp11.

Key words: bacteriophage T4, baseplate, gene product 11, gene product 10, deletion variants, protein folding, monoclonal antibodies

Bacteriophage T4 late gene product 11 (gp11) is a structural protein of the baseplate (BP), a complex molecular structure located on the distal end of the contractile tail and responsible for recognition, adsorption, and introduction of the phage's DNA into the *E. coli* cell. Bacteriophage T4 gene products gp11, gp10, gp6, gp7, gp8, gp10, gp25, and gp53 combine sequentially to assembly a wedge. Gp11 directly interacts with gp10 and with short tail fibers (gp12) [1, 2]. During infection, gp11 transmits a signal from the long tail fibers to the short ones which leads to structural reorganization of the BP and turn-down of the short fibers. This makes adsorption of a phage on the host cells surface irreversible and leads to subsequent tail contraction, which initiates DNA ejection [2].

Earlier we have developed a system of expression and purification of biologically active gp11 [3]. In collaboration with M. Rossmann's laboratory (Purdue University, USA), the three-dimensional structure of this protein was determined to 2.0 Å resolution [4]. According to the X-ray analysis data, the gp11 polypeptide chain containing 219 amino acid residues (aa) consists of three domains: the N-terminal domain (residues 1-64), the middle "finger" domain (residues 80-188), and the C-terminal domain generated by residues 65-79 and 189-219. The functionally active form of gp11 is a trimer.

The gp11 trimer with known atomic structure is a convenient model for studying the mechanisms of folding and oligomerization. It is worthwhile to establish the functional role of gp11 in transmitting a signal to the short tail fibers and in structural reorganization of the BP in order to understand the phage infection mechanism as a whole. Besides, the assembly of the BP wedge begins with formation of a complex of gp10 with gp11 during the phage particles morphogenesis *in vivo*.

Our goal was to study the role of the gp11 N-terminal domain in the formation of the functionally active trimer protein and in forming a complex with gp10. We have prepared a number of deletion variants of gp11 and gp10 and monoclonal antibodies (mAbs) specific to the native conformation of gp11. Using mAbs, it was possible to show that the removal of the first 17 amino acid residues of the

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gp11 polypeptide chain does not break the process of folding and trimerization of the protein, and truncated gp11 remains biologically active in a complementation system *in vitro*, but does not form a complex with gp10. More extended deletions in the N-terminal domain of gp11 do not influence the ability of the proteins to form trimers but significantly alter their structure, which leads to the loss of biological activity. These data suggest that the N-terminal fragment (residues 1-17) of gp11 interacts with the central region of the gp10 trimer during the formation of a complex of these two proteins *in vitro*.

MATERIALS AND METHODS

Bacterial strains. The *E. coli* TOP10 strain (Invitrogen, USA) was used for the plasmid DNA preparation and screening. The *E. coli* BL21(DE3) strain [5] was used for expression of genes cloned into plasmid vectors. The *E. coli* CR63 strain was used as a permissive host for the amber mutants in gene 11 of bacteriophage T4, and the *E. coli* $B^e/1$ strain was used as a restrictive host.

Expression of full-length gp11 was carried out as described earlier [3].

Cloning of gp11 fragments. Fragments of various lengths of gene 11 were prepared in polymerase chain reaction (PCR) using oligonucleotide primers with single oligonucleotide replacements for creation of corresponding sites of restriction. To obtain fragments of gene 11 coding proteins with deletions of 17, 35, 44, 63, and 120 N-terminal residues (NΔ17, NΔ35, NΔ44, NΔ63, NΔ120), the following forward primers were respectively used: 5′-GCCGATTCCATGGGTTTTAGA, 5′-CGTCAATCCATGGGGTCAGTGACAATA, 5′-CAATTAGCCATGGGATTTTAT, 5′-CATAATTTTTCCATGGAAGACGTT, 5′-GCAACAACTGCCATGGCGGCAATT; as the reverse primer, 5′-CTATCGGTAGGATCCAATTTTACA (*Nco*I and *Bam*HI restriction sites are underlined) was used. To obtain a PCR fragment of a gene coding a protein with a deletion of 28 C-terminal residues (CΔ28), following primers were used: forward 5′-CCGCCAACATCTAGAACTAAC, reverse 5′-ATTCCATGGACCTCAGCCAGGCTT. Fragments of DNA with a deletion of 5′-terminal domains of gene 11 cloned into the *Nco*I-*Bam*HI sites of pET23d(+) (Novagen, USA) vector, and a fragment of DNA with a deletion of 3′-terminal domain of gene 11 was cloned into the *Xba*I-*Nco*I sites of pET19b (Novagen) vector.

Cloning of gp10 and its fragments. The full-length gene 10 was amplified from phage T4 DNA using PCR using the oligonucleotide primers: 5′-GGGGTGGCTCACATATGAAACA (forward), 5′-ATTATTAAGTGAGCTCCTTATGC (reverse), and cloned into *Nde*I and *Sac*I sites of the pET22b(+) vector (Novagen). To obtain gene 10 fragments coding proteins (NΔ224, NΔ314, NΔ395) with 224, 314, and 395 N-terminal residues deletions, the following forward primers were respectively used:

5′-GAACTGGTTCCCATGGATGGATTTAAC,

5′-CAT TAT TAA CCA TGG ATG AGA TTA TTG ATG,

5′-TTC GTT CCA TGG GGA TTT TAC AGG AAT TGG CT

(*Nco*I sites are underlined); as reverse primer, the same oligonucleotide as for amplification of full-length gene 10 was used. Fragments of gene 10 were cloned into *Nco*I and *Sac*I sites of the pET23d(+) vector.

Expression of recombinant proteins in *E. coli* BL21 (DE3). Expression of genes cloned under the control of the bacteriophage T7 promoter was carried out according to the Studier method [5]. Competent cells BL21(DE3) after plasmid transformation were plated on 1.5% agar containing 100 μg/ml of ampicillin and incubated overnight at 37°C. The transformed cells were transferred to 2× TY media containing 200 μg/ml of ampicillin and grown at 37°C to A_{600} 0.5 under intensive aeration. Then the inducer of synthesis IPTG (isopropyl-1-thio-β-D-galactoside) was added to final concentration 1 mM to induce the expression, and the culture was incubated additionally for 3-4 h. The cells were centrifuged at 3500 rpm for 20 min (Megafuge 2.0 R, Heraeus Instruments, Germany).

Purification of recombinant proteins was carried out as described earlier for full-length gp11 [3].

Immunization of animals. Female mice (BALB/cJcitMouse, 14-16 g) were immunized with a suspension of recombinant gp11 as described earlier [6].

Production of hybridoma cells. Fusion of myeloma cells line Sp2 with splenocytes was carried out in the presence of 50% polyethylene glycol 4000 (PEG 4000). Clones producing mAbs were identified by direct enzyme-linked immunosorbent assay (ELISA) using purified gp11 as an antigen. Hybridomas were subcloned by the method of limiting dilutions [7].

Production of the ascites. To obtain large quantities of antibodies of interest, 24 h before the hybridoma cells were intraperitoneally injected into BALB/c mice (~10^6 cells per mouse), the animals were injected with 0.5 ml incomplete Freund's adjuvant. Ascites fluid was harvested from mice that developed a peritoneal tumor.

Purification of antibodies. The ascites fluid containing mAbs was purified by centrifugation (6000*g*, 15 min) and ammonium sulfate precipitation (50% saturated solution) with subsequent dialysis in phosphate-buffered saline (PBS).

Enzyme-linked immunosorbent assay (ELISA). ELISA was carried out as described earlier [6]. In direct ELISA, polystyrene microtiter plates (Costar, USA) were coated with purified gp11.

In competitive ELISA, microtiter plates were coated with full-length gp11. Cell extract containing gp11 or its deletion variants at concentration 1-2 mg/ml were mixed with equal volumes of the tested mAbs that were titrated by 2-fold dilutions in PBS containing 0.05% Tween-20 (PBS-T). After 1 h incubation at a room temperature, mAbs and antigen were mixed and transferred to gp11-coated plates. A solution of antibodies in PBS-T instead of a protein was used as a control. In a series of tests, the protein was denatured by heating for 15 min in a boiling water bath and then quickly diluted to the necessary concentration, inoculated on plates, and incubated with mAbs.

Western blot procedure was carried out as described earlier [6].

Antibody–antigen association rate constants in solution were determined as described in [10]. Each definition was carried out not less than five times.

Amber mutants of phage T4. An individual lysis colony of phage T4 amber mutation in gene 11 (amN93) on *E. coli* CR63 cells was extracted from top agar and transferred into 0.5 ml of physiological solution containing 20 mM $MgCl_2$ and incubated for 1 h at room temperature; this solution was used for infecting 5 ml of liquid culture of *E. coli* CR63 in exponential growth phase with $2 \cdot 10^8$ cells per ml with following incubation for 16 h at 30°C. The cells were lysed by adding two drops of chloroform. The debris was removed by centrifugation at 12,000 rpm for 10 min. The titer of infective particles was ~$5 \cdot 10^{10}$ plaque forming units per ml.

Preparation of cell extracts containing recombinant proteins. A 0.5-ml sample of cell culture was pelleted and resuspended in 100 µl of DMM buffer (Na_2HPO_4, 0.6 g/liter; KH_2PO_4, 0.3 g/liter; NaCl, 0.05 g/liter; NH_4Cl, 0.1 g/liter; 20 mM $MgCl_2$, pH 7.5) and incubated with lysozyme (0.3 mg/ml), DNase I (0.05 mg/ml), and phenylmethylsulfonyl fluoride (PMSF) (1 mM) for 15 min at room temperature. The mixture then was subjected to three cycles of freezing in liquid nitrogen (–70°C) with subsequent thawing in a water bath at 30°C. The lysate was centrifuged at 12,000 rpm for 2 min. Supernatant was prepared directly before the experiment.

Preparation of defective particles of phage T4. Cell extract containing 11^- particles of phage T4/11amN93 was prepared as described earlier [8]. *Escherichia coli* B^c/1 cells were grown in 50 ml of 2× TY medium containing 5 mM $MgCl_2$ at 37°C to a density of $2 \cdot 10^8$ cells/ml and infected by the amber mutant amN93 with multiplicity of 5. After incubation for 15 min at 30°C, the concentration of $MgCl_2$ was increased to 20 mM and the culture was additionally incubated for 40 min. The cells were pelleted at 4000 rpm for 10 min and resuspended in 2 ml of DMM containing 0.05 mg/ml DNase I. Aliquots were stored at –70°C.

Complementation assay *in vitro*. A 20-µl sample of the extract containing recombinant protein was mixed with 20 µl of cell lysate containing 11^- particles of phage T4. The mixture was incubated for 2 h at 30°C. During incubation, 5 µl aliquots were diluted 1000-fold in physiological solution and titrated using *E. coli* CR63 cells. To study the influence of deletion variants of gp11 on their completing of phage 11^- particles, a defective extract was preliminarily incubated for 30 min at 30°C with the cell extracts containing truncated proteins, and then full-length gp11 was added.

Electrophoresis of proteins in polyacrylamide gel. Electrophoresis was carried out according to the Laemmli method [9] and were stained with 0.3% Coomassie R (Sigma, USA) in acetic acid–ethanol–water (1 : 3 : 6 v/v) solution and destained with 50% ethanol and 7% acetic acid solution. As a marker, we used the Mark12 mix of proteins (Invitrogen) containing myosin (200 kD), β-galactosidase (116.3 kD), phosphorylase *b* (97.4 kD), bovine serum albumin (BSA) (66.3 kD), glutamic dehydrogenase (55.4 kD), lactate dehydrogenase (36.5 kD), carbonic anhydrase (31 kD), trypsin inhibitor (21.5 kD), lysozyme (14.4 kD), and aprotinin (6.0 kD) (Fig. 2) or protein mixture (Bio-Rad, USA) containing phosphorylase *b* (97.4 kD), serum albumin (66.2 kD), ovalbumin (45 kD), carbonic anhydrase (31 kD), and trypsin inhibitor (21.5 kD) (Fig. 7). Samples were prepared as follows: 100 µl sample of cell culture after expression was mixed with 30 µl of 4× sample buffer (0.25 M Tris-HCl, pH 6.8, 8% SDS, 40% (v/v) glycerol, 20% (v/v) 2-mercaptoethanol, 0.02% (w/v) bromophenol blue), heated for 5 min at 100°C, and then 10 µl were loaded on a gel.

Gel filtration. Analytical gel filtration of purified gp11 was carried out on a column (1.4 × 30 cm) packed with Superose 12 (FPLC, Pharmacia Biotech, Sweden) in 50 mM Tris-HCl buffer, pH 8.0, containing 1 mM EDTA with flow rate 0.2 ml/min. The column was calibrated with albumin (67 kD) and chymotrypsinogen A (25 kD).

RESULTS

Production of deletion mutants of gp11. According to the X-ray analysis data, the N-terminal domain of gp11 contains three short α-helical sections of different lengths connected by loops (the first 10 residues of polypeptide chain in the protein crystal are not ordered) (Fig. 1). The first two α-helices do not interact with each other, whereas the most extended α-helices of monomers (residues 12-64) form a coiled-coil structure in the trimer. We have designed plasmid vectors for expression of gp11 deletion variants: NΔ17, NΔ35, NΔ44, NΔ63, and NΔ120, shortened by 17, 35, 44, 63, and 120 amino acid residues, accordingly, from the N-terminus of the polypeptide chain. We have also created the expression vector for the mutant CΔ28 with the deletion of 28 amino acid residues from the C-terminal domain, which forms two β-sheets. A

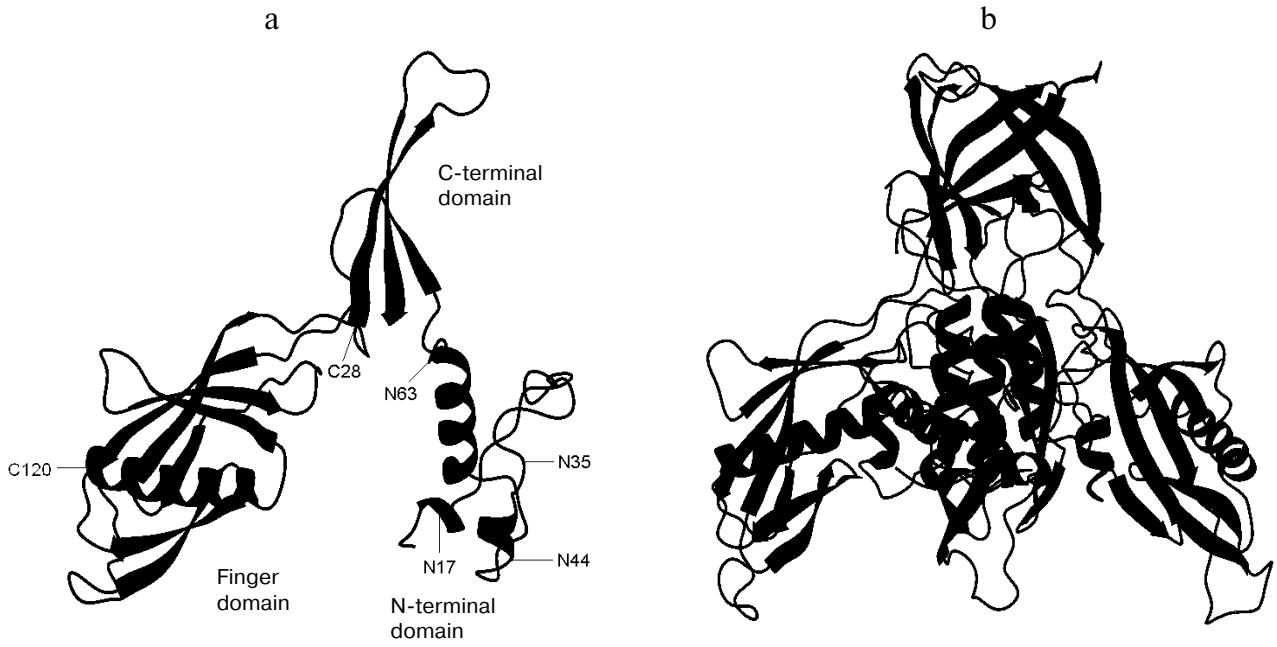

Fig. 1. Crystal structure of gp11 determined to 2.0 Å resolution [4]: a) gp11 monomer; b) trimer. Truncated residues from N- or C-terminus of polypeptide chain are labeled.

high level of expression was typical for all expression vectors, which was shown by major bands of corresponding proteins after separation on SDS-PAGE cell BL21(DE3) lysates (Fig. 2). Proteins CΔ28 and NΔ120 formed aggregates, and the other N-terminal deletion mutants of gp11 were soluble. For further work, NΔ17, NΔ35, NΔ44, and NΔ63 proteins were prepared in preparative amounts and purified to homogeneous condition.

Biological activity of the mutant proteins. As shown earlier, recombinant gp11 is able to incorporate into 11^- phage particles making them infectious [3]. The biological activity analysis in a complementation system *in vitro* was carried out with deletion variants of gp11: NΔ17, NΔ35, NΔ44, and NΔ63 (table). For the NΔ17 mutant, as well as in the case of the full-length gp11, the phage titer increased by almost three orders in comparison with the negative control, which suggests that the protein remained active. Proteins with more extended deletions in the N-terminal domain (NΔ35, NΔ44, NΔ63) did not restore biological activity of the defective particles (table). Moreover, the results of competitive complementation with NΔ35, NΔ44, and NΔ63 showed that the given

Titer of T4 infection particles in *in vitro* complementation of phage 11^- particles with full-length gp11 and its mutants

Recombinant protein	Phage particle titer in direct complementation (plaque forming units per ml)	Phage particle titer in competitive complementation with subsequent addition of full-length gp11 (plaque forming units per ml)
— (negative control)	$(4.6 \pm 0.5) \cdot 10^8$	—
Full-length gp11 (positive control)	$(1.7 \pm 0.4) \cdot 10^{11}$	—
NΔ17	$(1.9 \pm 0.5) \cdot 10^{11}$	$(1.7 \pm 0.5) \cdot 10^{11}$
NΔ35	$(4.5 \pm 0.3) \cdot 10^8$	$(1.3 \pm 0.6) \cdot 10^{11}$
NΔ44	$(4.1 \pm 0.2) \cdot 10^8$	$(1.4 \pm 0.5) \cdot 10^{11}$
NΔ63	$(5.0 \pm 0.6) \cdot 10^8$	$(1.8 \pm 0.4) \cdot 10^{11}$

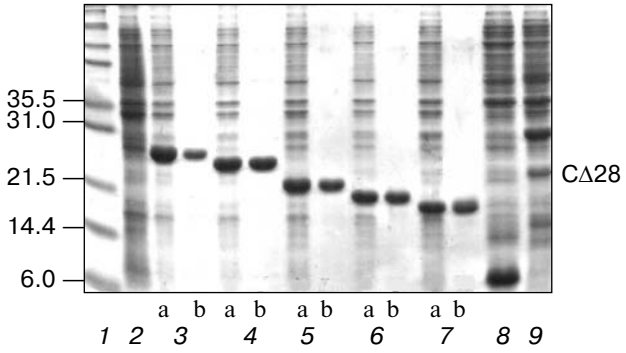

Fig. 2. Electrophoretic analysis in 15% SDS-polyacrylamide gel of the recombinant gp11 after expression (a) and purification (b): *1*) marker proteins; *2*) *E. coli* cell lysate before induction; *3*) gp11; *4*) NΔ17; *5*) NΔ35; *6*) NΔ44; *7*) NΔ63; *8*) NΔ120; *9*) CΔ28.

mutants do not influence the ability of full-length protein to restore biological activity of phage 11⁻ particles. This means that they are not able to incorporate into the phage BP. Apparently, the removal from 35 up to 63 amino acid residues leads to significant changes in protein structure and loss of biological activity.

Thus, deletion of at least 17 N-terminal amino acid residues is insignificant for functioning of gp11, incorporated into phage BP *in vitro*, but deletion from 35 residues until full removal of the N-terminal domain leads to a full loss of activity of these mutant proteins.

Trimerization of gp11 deletion fragments. Gel filtration of the purified recombinant proteins was carried out to study the influence of gp11 N-terminal domain deletion on trimer formation. Deletion variants NΔ17, NΔ44, and NΔ63 were eluted from a column in the volumes corresponding to their trimer forms (Fig. 3). Thus, deletions in the N-terminal domain of gp11 do not influence its ability to trimerize.

Analysis of gp11 deletion variants with mAbs. The analysis of complementation test data showed that biologically active variant of protein NΔ17 keeps its tertiary and quaternary structure native, whereas loss of complementation ability of mutant proteins NΔ35, NΔ44, and NΔ63 *in vitro* is apparently connected with significant conformational changes in their structure.

Monoclonal antibodies are sensitive tools for studying changes of protein structure on introduction of mutations including deletions [6, 10, 11]. For this purpose, we have prepared a series of hybridoma clones producing mAbs that recognize full-length gp11. Using competitive ELISA, we determined the ability of the given antibodies to recognize native and heat-denatured form of gp11. For further research, three mAbs (5F9, 2D2, 4A8), which interact with the native conformation of gp11 and recognize different sites of the molecule, were selected. Affinity constants of 5F9 and 4A8 in complex with native gp11 are approximately four orders higher than in complex with

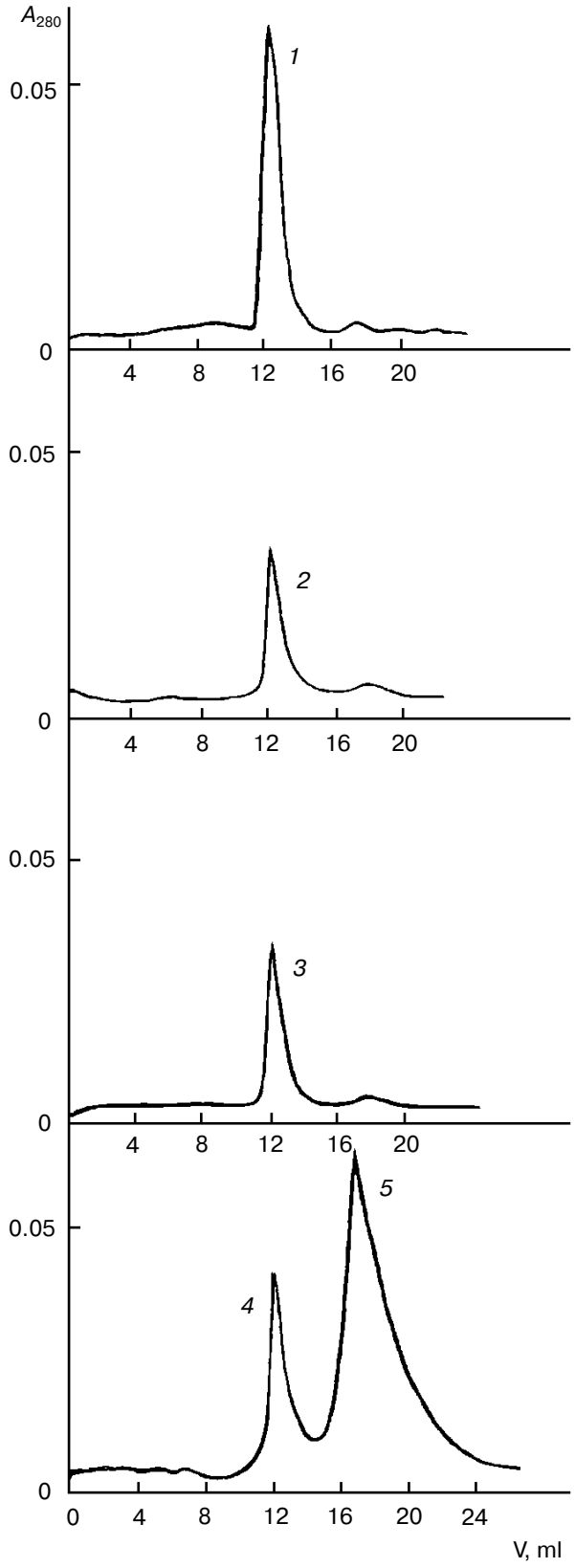

Fig. 3. Gel filtration of gp11 deletion variants on Superose 12: NΔ63 (*1*), NΔ44 (*2*), NΔ17 (*3*), mixture of albumin (*4*) and chymotrypsinogen A (*5*). Flow rate is 0.2 ml/min.

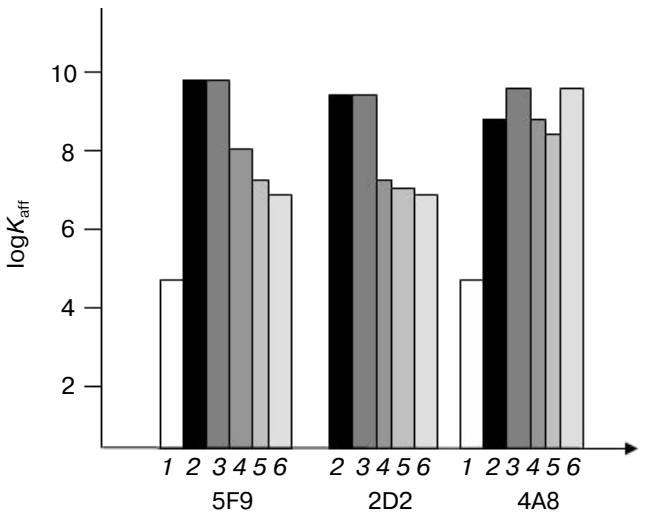

Fig. 4. Association rate constants of 5F9, 2D2, and 4A8 mAbs with recombinant variants of gp11 (from ELISA data): *1*) denatured gp11; *2*) native gp11; *3*) NΔ17; *4*) NΔ35; *5*) NΔ44; *6*) NΔ63.

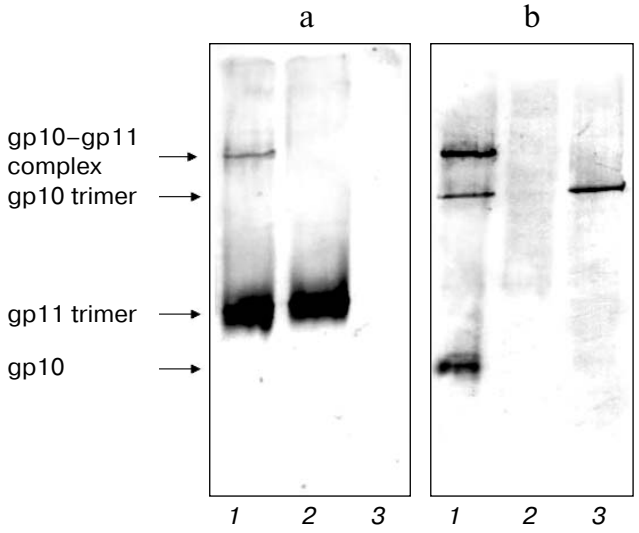

Fig. 6. Immunoblotting of recombinant proteins under native conditions in 10% polyacrylamide gel: *1*) gp11 and gp10 mix; *2*) full-length gp11; *3*) gp10. Detection with mAb 2D2 (a) and polyclonal antibody against gp10 (b).

denatured gp11. Apparently, the 2D2 antibody is a conformational one as it recognizes only native gp11 and does not interact with its denatured form (Fig. 4, columns *1* and *2*).

The ability of mAbs to recognize the full-length gp11 native conformation was used to study its deletion variants in competitive ELISA (Fig. 4, columns *3-6*). mAbs did not reveal any differences in the gp11 full-length and its deletion fragment NΔ17 structures. However more extended deletions lead to a significant structure alteration of gp11 sites recognized by 5F9 and 2D2 antibodies, which was indicated by a 2-3 order decrease in affinity constants of these mAbs with NΔ35, NΔ44, and NΔ63 in comparison with native gp11. At the same time, removal of the N-terminal domain did not reveal a significant change in an epitope recognized by 4A8 antibody (Fig. 4).

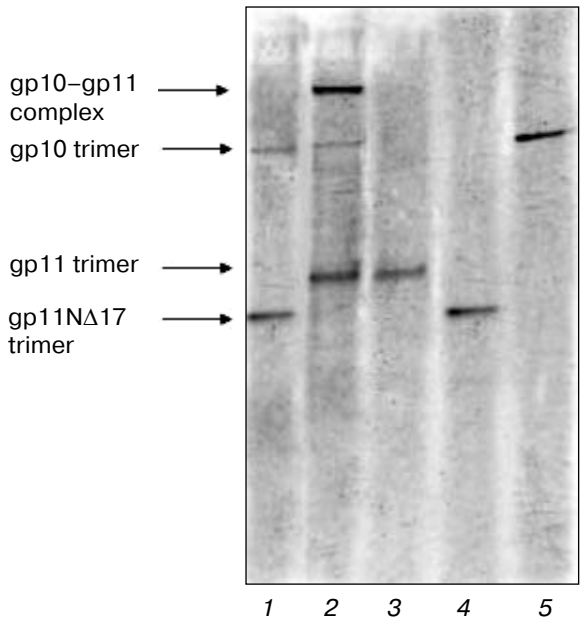

Fig. 5. Electrophoretic analysis in 10% polyacrylamide gel of the recombinant proteins: *1*) NΔ17 and gp10 mix; *2*) gp11 and gp10 mix; *3*) full-length gp11; *4*) NΔ17; *5*) gp10.

Gp10–gp11 complex formation. In an earlier work [12], it was shown that during the assembly of a phage particle gp11 interacts with gp10. Therefore, we had to check the ability of recombinant gp10 and gp11 to form a complex *in vitro*. For this purpose cell extracts containing these proteins were mixed at the ratio 1 : 1 and incubated for 1 h at 4°C and then analyzed by electrophoresis under native conditions (Fig. 5) and immunoblotting (Fig. 6). As seen in the figures, gp10 forms a complex with full-length gp11. Gp11 lacking 17 amino acid residues at its N-terminus does not form a complex with gp10. Apparently, this site of gp11 is essential for the formation of a stable complex with gp10 *in vitro*. It is possible that this interaction is realized in the process of BP wedge assembly *in vivo* during T4 phage particle morphogenesis.

Localization of the interaction site in the gp10 molecule with gp11. As established earlier [13], the stoichiometric ratio of proteins in the gp10–gp11 complex is 3 : 3, and gp10 (602 amino acid residues) also, as well as gp11, is a trimer. In contrast to gp11, recombinant gp10 forms a stable trimer in the presence of SDS, its band being visible on a gel in the analysis of an unheated cell extract

sample after expression (Fig. 7, lanes *1*, a and b). The gp10 C-terminal domain is apparently responsible for its trimerization since the mutant molecules shortened from the N-terminus (NΔ224 and NΔ314), similarly to the full-length gp10, form stable trimers in the presence of SDS (Fig. 7, lanes *2* (a, b) and *3* (a, b)). More extended deletion (mutant NΔ395) apparently breaks the process of trimerization (or an unstable trimer is formed in the presence of SDS). In the case of C-terminal deletions of the gp10 polypeptide chain, there are no stable protein trimers in the presence of SDS (data not shown).

We have studied the ability of gp10 mutant variants to interact with full-length gp11 in solution. Appearance of an additional band corresponding to a complex of the two proteins is observed only for NΔ224 mutant (Fig. 8, lane *2*), whereas in other cases (Fig. 8, lanes *3* and *4*) there are bands corresponding to the individual proteins. Probably, the central region of gp10 (residues 225-315) is responsible for interaction with gp11. More detailed information about the sites of interaction of these two proteins and their interaction as a part of the phage BP will be obtained after the determination of the crystal structure of the gp10–gp11 complex.

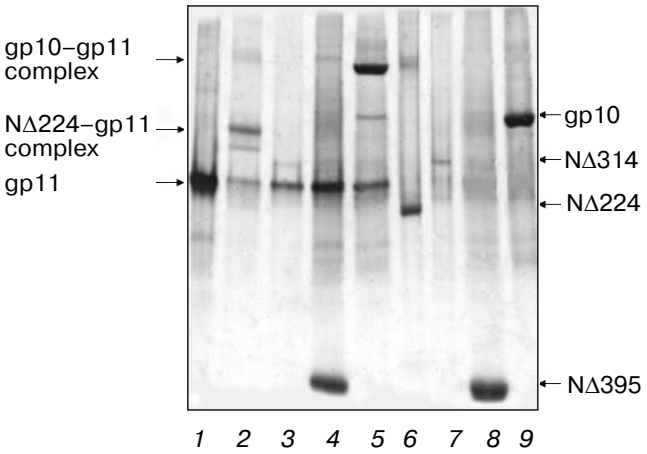

Fig. 8. Electrophoretic analysis in 10% SDS-polyacrylamide gel of gp11 complexes with gp10 and its deletion variants: *1*) gp11; *2*) incubation mix of gp11 with NΔ224; *3*) incubation mix of gp11 and NΔ314; *4*) incubation mix of gp11 and NΔ395; *5*) incubation mix of gp11 and full-length gp10; *6*) NΔ224; *7*) NΔ314; *8*) NΔ395; *9*) full-length gp10.

DISCUSSION

Summarizing the data of gp11 deletion variant properties, it is possible to conclude that the folding of its variants lacking 17 N-terminal residues proceeds correctly, leading to assembly of a functionally active trimer capable of complementation *in vitro*. More extended deletions up to full removal of its N-terminal domain do not influence its ability to trimerize, but however significantly alter its structure and therefore the mutant proteins completely lose their biological activity.

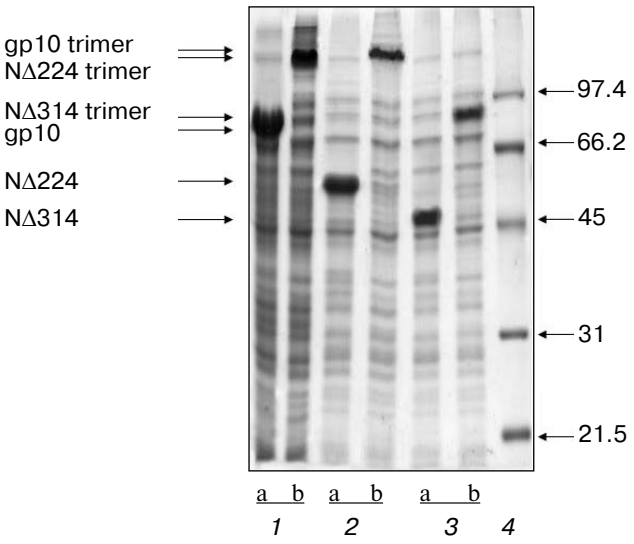

Fig. 7. Electrophoretic analysis in 10% SDS-polyacrylamide gel of the recombinant proteins after expression ((a) heated samples; (b) unheated samples): *1*) full-length gp10; *2*) NΔ224; *3*) NΔ314; *4*) marker proteins (on the right, their molecular weights in kD).

As shown *in vitro*, the N-terminal site gp11 trimer is necessary for interaction with gp10. On the basis of the properties of gp10 deletion variants, it is possible to assume that the central region of its trimer is responsible for interaction with gp11. This interaction is apparently essential for phage BP assembly *in vivo*.

It is also important to understand how mutual orientation of the given proteins in the BP changes throughout transformation of its structure from "hexagonal tent" to "star" during infection. The gp11 trimer associates with the short tail fibers and a domain of gp10 is clamped between the three fingers of gp11. The kink of each fiber is attached to the space between the central head domain and one of the fingers of the gp11 trimer [1]. In the phage particle where the BP has the tent form, its stability is maintained by the interaction of short tail fibers with each other and with gp11 [14].

The three-dimensional reconstruction [2] has shown that some proteins change their position during infection and conformational changes of the phage BP. Thus, the major arrow-like domain of gp10 (80 Å) changes its orientation from being at ~45° with respect to the baseplate sixfold axis to nearly orthogonal to it. Compared to the native conformation, these rearrangements lead to flattening and widening of the star-shaped baseplate [2]. The proximal part of the long tail fibers emanates from the star-shaped baseplate; long tail fibers are attached collinearly with gp9 and have also a lateral interaction with gp11, removing it from short tail fibers. The orienta-

tion of the gp11 trimer also changes. In the native BP, the gp11 trimer axis forms an ~144° angle with respect to the baseplate sixfold axis; in the star-shaped structure, the gp11 axis has ~48° angle. Therefore, our further research will focus on the study of gp11 conformational changes in the process of infection using available mAbs to that protein.

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