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Properties of Bacteriophage T4 Baseplate Protein Encoded by Gene 8

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Abstract—Gene product 8 (gp8, 344 amino acids per monomer) of bacteriophage T4 is one of the baseplate structural proteins. We constructed an expression vector of gp8 and developed a method for purification of recombinant protein. CD spectroscopy showed that gp8 is an α/β type structural protein. Its polypeptide chain consists of nearly 40% β-structure and 15% α-helix. These data agree with results of prediction of secondary structure based on the amino acid sequence of the protein. The sedimentation coefficient under standard conditions ($S_{20,w}$) is 4.6S. Analytical ultracentrifugation results demonstrated that gp8 in solution has two types of oligomers—dimer and tetramer. The tetramer of gp8 may be included in the wedge (1/6 of the baseplate), and the dimer may be an intermediate product of association.

Key words: bacteriophage T4, baseplate, gene product 8, CD spectroscopy, α/β proteins

The bacteriophage T4 baseplate is a hexagonal disk located on the end of a tail of a viral particle. During infection, the baseplate functions in the interaction and recognition of the host cell and also in the adsorption (long and short tail fibers) and DNA introduction into the cytoplasm of the cell (the tail sheath). During infection, the baseplate undergoes global structural transformation, changing from a hexagon to a 6-fold star [1].

Under native conditions the baseplate has 6th order symmetry and consists of 6 identical protein complexes, so-called wedges, and a central hub. A wedge is a product of consecutive assembly and connection of seven proteins (gp10, gp7, gp8, gp6, gp25, gp53, gp11) [2, 3]. In the following step of assembly, 6 wedges join around of the central hub and the formed complex is stabilized by the connection of two more proteins, gp9 and gp12, the short tail fiber, providing irreversible viral adsorption onto host cells [4]. To understand the structure of the baseplate and details of conformational changes during infection, we investigate separate proteins and intermediate complexes of the baseplate assembly [5, 6]. We have determined the X-ray structure of one protein of the wedge, gp11 [5], and the structure of gp9 [6], the connector of long tail fibers, which is located in the corners of the baseplate.

Abbreviations: PMSF) *o*-phenylmethylsulfonyl fluoride; SDS) sodium dodecyl sulfate; IPTG) isopropyl β-D-thiogalactopyranoside; PCR) polymerase chain reaction; gp) gene product.

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It was earlier established that gp8 is located inside a wedge and directly contacts with gp7 and gp6 [7-9]. The genes coding proteins of the baseplate have been known for a long time [1, 2], but the exact numbers of copies of each have not yet been established. It was recently found that the structure of a wedge includes three copies gp10 and gp11 [10]. The data in the literature on gp8 varies from one to three copies per wedge [1, 3, 7, 8]. We developed a system for expression of gp8, cloned in a plasmid, and studied the structure of this protein by circular dichroism spectroscopy and analytical ultracentrifugation.

MATERIALS AND METHODS

Gp8 cloning. The DNA sequence of gene 8 was first determined in our laboratory [11]. A DNA fragment containing gp8 was amplified by PCR according to a standard procedure [12]. T4 phage DNA was used as the PCR matrix. The sequence of PCR primers used were:

Gp8-fwd 5′-GAAAATCCAACACAGGTTAAAAT*CCATGG*-3′

and

Gp8-rev 5′-CCAT*GAGCTC*CCTAAATTTAAAATGT-3′.

The primers contained sites of restriction (shown in italics) *Nco*I and *Sac*I, respectively, which were used for

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cloning. An amplified DNA fragment was purified using a QiaEx kit (QiaGen, Germany). The DNA fragment was cloned into pET23d(+) (Novagen, USA) vector under control of the T7 promoter. The clone selection and DNA isolation were according to standard protocols [12].

Protein expression. *E. coli* BL21(DE3) cells infected by a plasmid (pET23/g12) were grown in 2xYT medium with ampicillin (200 μg/ml) at 37°C to $A_{600} = 0.6$, then the synthesis of recombinant protein was induced by addition of IPTG to final concentration 1 mM [13] and incubation was continued for 3 h with intensive aeration at 37°C. The cells were harvested by centrifugation at 3000*g* for 15 min at 4°C.

Protein purification. Pelleted *E. coli* cells from 1 liter of medium were resuspended in 20 ml of buffer containing 50 mM Tris-HCl, pH 8.0, 50 mM NaCl, 2 mM EDTA, and 1 mM PMSF. Cell suspension was sonicated (6 bursts × 30 sec, at 4°C, Techpan TD-20). Cell debris was pelleted at 30,000*g* for 15 min at 4ºC (Sorvall JA-20 rotor). Streptomycin sulfate was added to the cell extract to final concentration 3%, and the sample was kept 20 min at 4°C and then centrifuged at 30,000*g* for 15 min at 4°C. After separation of nucleic acid, the protein was precipitated by addition of ammonium sulfate to 24-26% saturation and subsequent incubation for 8 h at 4°C, then the protein was pelleted by centrifugation at 30,000*g* for 15 min at 4°C. The protein precipitate was dissolved in 50 mM Tris-HCl, pH 8.0, and dialyzed against 1 liter of the same buffer for 20 h.

The supernatant was applied to an anion-exchange column with Fractogel® EMD TMAE-650(S) (EM Separations Technology, USA), and the protein was eluted with a 50-400 mM gradient of NaCl in 50 mM Tris-HCl, pH 8.0, 2 mM EDTA. The protein of interest was eluted at 200-300 mM NaCl. Fractions containing the protein were pooled, PMSF added to final concentration 1 mM, and the protein dialyzed against 1 liter of 10 mM Tris-HCl, pH 7.5, 2 mM EDTA for 20 h.

The protein was applied on a column with hydroxyapatite (BioRad, USA) and eluted with a gradient of sodium phosphate, pH 7.5. Gp8 was eluted at 10 mM sodium phosphate. The fractions containing gp8 were pooled, PMSF was added to 1 mM, and the protein was dialyzed against 10 mM Tris-HCl, pH 7.5, 2 mM EDTA. The protein was stored at 4°C.

Analytical ultracentrifugation. Sedimentation velocity and sedimentation equilibrium runs of gp8 were performed in a Beckman XLA analytical ultracentrifuge (Beckman Instruments, USA) equipped with UV absorption optics and a photoelectric scanning system. Sedimentation velocity runs were performed at 20°C and 54,000 rpm in a 12-mm double sector cell. The absorbance was measured at 280 nm.

Sedimentation equilibrium runs were performed using the same cell but filled only to a level of about 2-3 mm at protein concentrations 0.25, 0.5, and 1.2 mg/ml in buffer PBS (10 mM sodium phosphate, pH 7.2, 150 mM NaCl) at 20°C for 20 h. Measurements were carried out at speeds of rotation 10,000, 14,000, and 16,000 rpm. At 10,000 rpm we recorded the higher molecular mass species and at 16,000 rpm the lower ones. The molecular masses were evaluated using a floating baseline computer program that adjusts the baseline absorbance to obtain the best linear fit of $\ln A$ versus r^2 (A, absorbance; r, radial distance).

Circular dichroism spectroscopy (CD). CD spectra of mutant proteins were recorded with an Aviv 62DS (Aviv Associates, Inc., USA) circular dichroism spectrometer equipped with a thermostatted quartz cell having a 1-mm path length at 25°C. Standard programs were used for evaluation of structural content [14-16].

Electrophoresis of gp8. SDS-PAGE was carried out according to the method of Laemmli [12]. Electrophoresis under at non-denaturing conditions was carried out by the same technique in 8% polyacrylamide gel but SDS was excluded from gel, buffer, and samples.

RESULTS AND DISCUSSION

Gp8 cloning and expression. Gene 8 of T4 phage was cloned into pET23d(+) vector by *Nco*I–*Sac*I sites. SDS-PAGE results show (Fig. 1, lane *2*) that production of gp8 in *E. coli* BL21(DE3) cells after 3 h of expression exceeds the amount of any host proteins.

Gp8 purification and electrophoresis. Recombinant gp8 of 95% purity was obtained by the purification process (Fig. 1, lane *6*). The purification of gp8 includes two main step: chromatography on anion-exchange resin

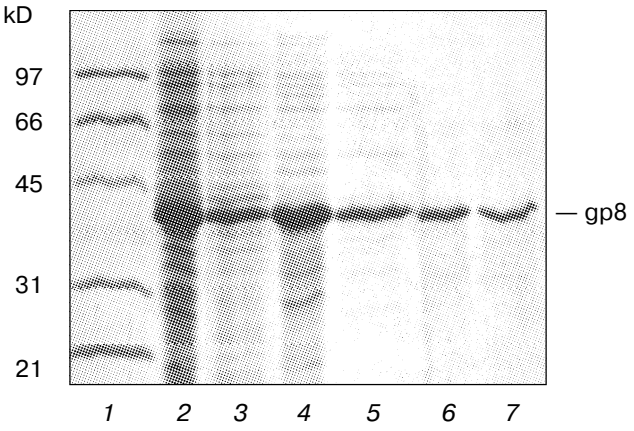

Fig. 1. Analysis of recombinant gp8 on 12% SDS-polyacrylamide gel in the process of purification: *1*) Low Range molecular weight marker kit (BioRad, USA); *2*) *E. coli* cell lysate after 3-h expression; *3*) lysate after precipitation of cell debris; *4*) cleared lysate after precipitation of nucleic acids with streptomycin sulfate; *5*) gp8 after chromatography on Fractogel® EMD TMAE-650(S); *6*) purified gp8 after chromatography on hydroxyapatite; *7*) not preheated gp8.

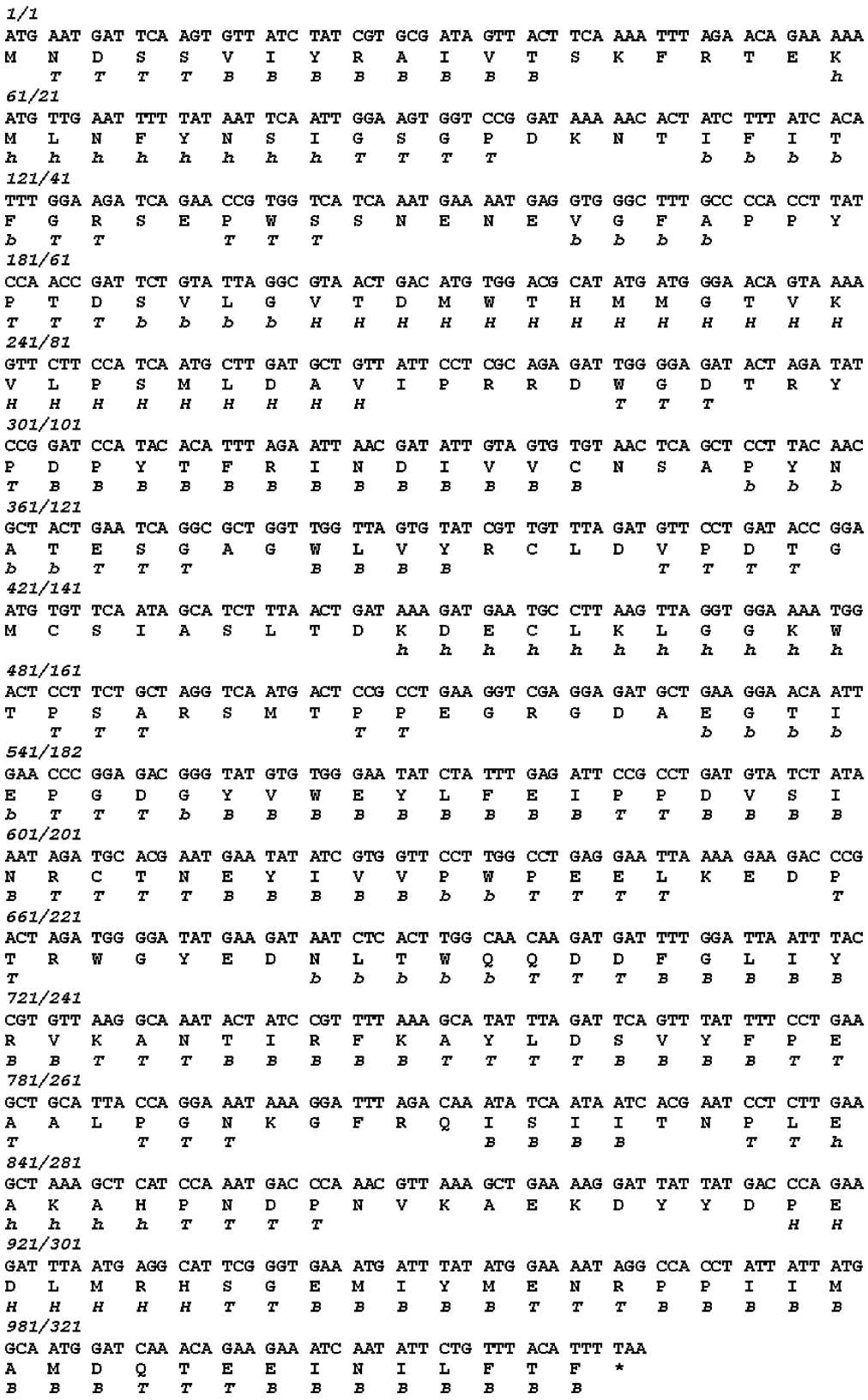

Fig. 2. DNA and amino acid sequences of gp8 with predicted secondary structure. Secondary structure elements: *H*) α-helix, high probability; *h*) α-helix, low probability; *B*) β-structure, high probability; *b*) β-structure, low probability; *T*) probable turns.

Results of calculation of secondary structure of gp8

Method	α	β	β_t	R
Provencher (1981)	0.17	0.41	0.21	0.21
Vassilenko (1993)	0.18	0.35	0.17	0.30
Sreerama (1994)	0.14	0.37	0.27	0.22

Fractogel® EMD TMAE-650(S) and subsequent chromatography on hydroxyapatite.

Recombinant gp8 preheated at 95°C for 5 min showed electrophoretic mobility of 38 kD by SDS-PAGE (Fig. 1, lane *6*). These data correspond with the molecular weight calculated from the amino acid sequence. The unheated protein had the same mobility as the heat-denatured sample (Fig. 1, lane *7*). This demonstrates SDS-sensitivity of the gp8 oligomer, unlike another T4 baseplate protein, gp9, which is an SDS-tolerant trimer [17].

Study of gp8 secondary structure. The secondary structure of the protein was predicted by the program ALB [18] according to the calculated amino acid sequence. The calculated amino acid sequence of gp8 with predicted elements of secondary structure is presented in Fig. 2. According to these data, the predicted β-structural content of gp8 structure (25%) prevails above α-helix (14%).

The CD spectrum of purified gp8 is presented in Fig. 3. The results of calculation of secondary structure of gp8 with three different methods [14-16] are presented in the table. The calculated data show relative higher contents of β-structure (35-41%) and moderate amounts of α-helix (14-18%). These data are in good agreement with

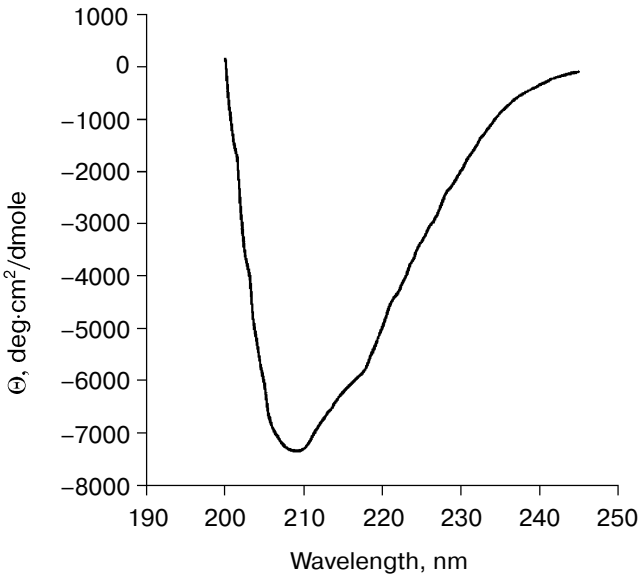

Fig. 3. CD spectrum of gp8.

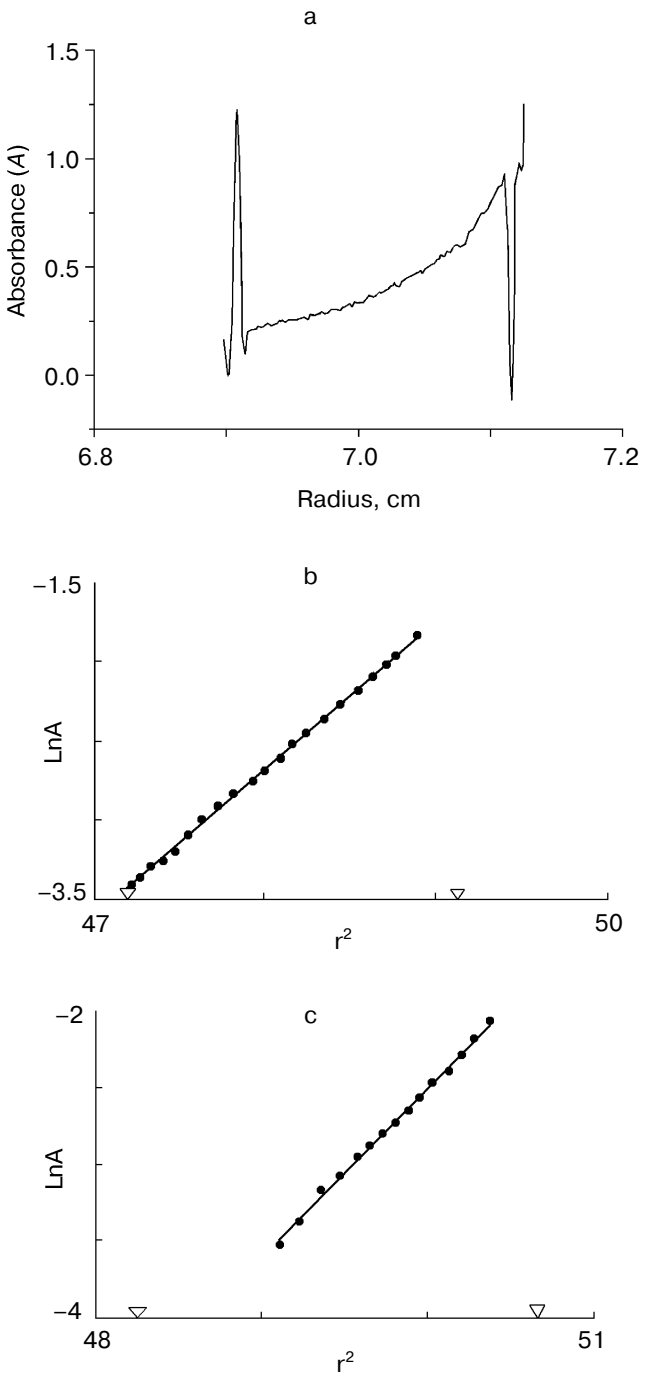

Fig. 4. Analytical ultracentrifugation of gp8. Sedimentation equilibrium data were ransformed to a ln*A* versus r^2 plot (*A*, absorbance; *r*, radial distance) (b, c). a) Sedimentation equilibrium data set at rotor speed 10,000 rpm shown as diagram *A* versus *r*; b) sedimentation equilibrium data transformation of data obtained at rotor speed 10,000 rpm; resulting molecular weight of oligomer, 154 kD; c) sedimentation equilibrium data transformation of data obtained at rotor speed 16,000 rpm; resulting molecular weight of oligomer, 72 kD.

the results of prediction of secondary structure of the protein based on its sequence as described above. Thus, gp8 has α-β structural types of fibers like gp9 and gp11, whose x-ray structure was determined by us earlier.

Study of gp8 oligomerization. Oligomerization degree of gp8 was determined by analytical ultracentrifugation. The sedimentation coefficient of gp8 under standard conditions ($S_{20,w}$) is 4.6S. Equilibrium experiments performed at rotor speeds of 10,000 and 16,000 rpm demonstrated the presence of oligomers with mass 155 and 72 kD (Fig. 4), respectively. Equilibrium centrifugation at intermediate speed of 14,000 rpm revealed both complexes (72 and 155 kD) in approximately equal amounts. Based on the molecular weight of the gp8 monomer, 38.0 kD, we calculated that gp8 in solution has two oligomers—dimer and tetramer.

Electrophoresis of pure gp8 under non-denaturing conditions demonstrated two bands of different mobility. The band of higher mobility may be the dimer of gp8, and the band of lower mobility may be the tetramer of the protein (data are not shown).

Earlier, electrophoresis of intermediates of a wedge assembly *in vitro* [3, 7] and the analysis of products of baseplate cross-linking [8, 9] have demonstrated the presence two or three copies of gp8 per wedge. Our data indicate that the wedge may contain four copies of gp8.

Within a wedge of the baseplate, gp8 directly contacts one copy of gp7 and two copies of gp6 [8, 9]. The symmetric model of the baseplate, where two copies of gp8 contact each molecule of gp6, is the simplest from the point of view of protein–protein interactions.

Now, in cooperation with the laboratory of M. Rossmann (Purdue University, USA), we have obtained crystals of gp8 for solution of its X-ray structure.

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REFERENCES

1. Crowther, R. A., Lenk, E. V., Kikuchi, Y., and King, J. (1977) *J. Mol. Biol.*, **116**, 489-523.
2. Kikuchi, Y., and King, J. (1975) *J. Mol. Biol.*, **99**, 645-672.
3. Ferguson, P. L., and Coombs, D. H. (2000) *J. Mol. Biol.*, **297**, 99-117.
4. Kikuchi, Y., and King, J. (1975) *J. Mol. Biol.*, **99**, 695-716.
5. Leiman, P. G., Kostyuchenko, V. A., Shneider, M. M., Kurochkina, L. P., Mesyanzhinov, V. V., and Rossmann, M. G. (2000) *J. Mol. Biol.*, **301**, 975-985.
6. Kostyuchenko, V. A., Navruzbekov, G. A., Kurochkina, L. P., Strelkov, S. V., Mesyanzhinov, V. V., and Rossmann, M. G. (1999) *Structure Fold. Des.*, **7**, 1213-1222.
7. Plishker, M. F., Rangwala, S. H., and Berget, P. B. (1988) *J. Virol.*, **62**, 400-406.
8. Watts, N. R., and Coombs, D. H. (1989) *J. Virol.*, **63**, 2427-2436.
9. Watts, N. R., and Coombs, D. H. (1990) *J. Virol.*, **64**, 143-154.
10. Zhao, L., Takeda, S., Leiman, P. G., and Arisaka, F. (2000) *Biochim. Biophys. Acta*, **1479**, 286-292.
11. Efimov, V. P., Prilipov, A. G., and Mesyanzhinov, V. V. (1990) *Nucleic Acids Res.*, **18**, 5313.
12 Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) *Molecular Cloning: a Laboratory Manual*, Cold Spring Harbor Press, New York.
13. Studier, F. W., Rosenberg, A. N., Dunn, A. H., and Dubendorff, J. W. (1990) *Meth. Enzymol.*, **185**, 60-69.
14. Provencher, S. W., and Glockner, J. (1981) *Biochemistry*, **20**, 33-37.
15. Venyaminov, S. Yu., and Vassilenko, K. S. (1994) *Anal. Biochem.*, **222**, 176-184.
16. Sreerama, N., and Woody, R. W. (1994) *J. Mol Biol.*, **242**, 497-507.
17. Navruzbekov, G. A., Kurochkina, L. P., Kostyuchenko, V. A., Zurabishvili, T. G., Venyaminov, S. Y., and Mesyanzhinov, V. V. (1999) *Biochemistry* (Moscow), **64**, 1266-1272.
18. Ptitsyn, O. B., and Finkelstein, A. V. (1983) *Biopolymers*, **22**, 15-25.