Number of Polypeptide Components in Bacteriophage T2L Contractile Sheaths

S. A. A. FARID1 and L. M. KOZLOFF

Department of Microbiology, University of Colorado Medical School, Denver, Colorado 80220

Received for publication 2 January 1968

Isolated purified contractile tail sheaths of bacteriophage T2L were analyzed for their carboxyl terminal amino acids by carboxypeptidase treatment and hydrazinolysis. Glycine and serine were identified as the only two carboxyl end groups. Using corrections for the yields of these two amino acids upon hydrazinolysis, we calculated that there are 154 (±30) moles of C-terminal glycine and 130 (±45) moles of C-terminal serine per mole of sheath. It appears likely that sheaths contain two types of polypeptide chains in equal numbers, probably 144 of each. The relation of these two components to the mechanism of sheath contraction is discussed.

In 1959, Kozloff and Lute pointed out that the main tail component of bacteriophage T2L had contractile properties (13). A number of properties of this viral component, in particular its dimensional changes during contraction, were investigated, and some similarities between this protein and other contractile systems were noted (9). It was found that phage preparations contained significant amounts of nucleoside triphosphates, and the addition of adenosine triphosphate (ATP) was shown to have a relaxing effect upon contracted tail sheaths. Later in 1959, Brenner and his colleagues (7) investigated the structure of the T-even bacteriophages. They also noted that the major tail protein had contractile properties and named this substructure the contractile sheath. They reported a simple method for isolating contracted sheaths and obtained high-resolution electron micrographs of negatively stained sheath preparations which revealed considerable detail of sheath fine structure.

This structure has since been widely investigated by physical and biochemical techniques since its mechanism of conformational change might bear some relationship to similar conformational changes in other systems. One approach to this problem has been taken by workers who have relied extensively on the appearance in the electron microscope of negatively stained uncontracted and contracted sheaths, as well as "poly-sheaths" (2, 5, 12, 16, 17). More recently, Krimm and Anderson (15) have applied optical diffractometry to analyze electron micrographs of contracted tail sheaths. These physical investigations have offered considerable insight into the nature of the conformational change of the sheath structure. Correlated with these observations have been chemical studies also concerned with this mechanism by Kozloff and his colleagues (9, 13, 14, 19, 20) and Polglasov (18). These later studies have described some chemical features of the composition of the isolated sheath and of its reaction with ATP.

A critical point in understanding the conformational change of this structure involves knowing the number of components which make up the tail sheath structure. The isolated sheath has a particle weight of 8 × 10^6 daltons (19) and is composed of many protein subunits. The first electron micrographs suggested that there were 144 large subunits, arranged in 24 annuli of 6 subunits each in the uncontracted sheath and in 12 annuli of 12 subunits each in the contracted sheath (7). It was also found that each sheath contained 144 histidine residues (19). Various earlier workers tentatively assumed that there is only one kind of protein subunit making up the tail sheath; presumably, each subunit would represent 1/144 of the total mass and would have a molecular weight of 55,000 daltons (7, 19). Bradley in 1963 (5) first proposed that there may be more than one kind of subunit in the sheath, and recently Moody (16, 17) has interpreted certain features of electron micrographs to support the view that there are two types of morphological subunits, one relatively large and one

1 Present address: S. A. A. Farid (Mrs. Sohair A. Sabet), U.S. Naval Medical Research Unit No. 3, Abbassiah, Cairo, United Arab Republic.
POLYPEPTIDES IN T2L CONTRACTILE SHEATHS

smaller, making up the tail sheath. Presumably, there would be 144 of each type per sheath, although in Moody's model these morphological subunits could still be part of one polypeptide chain.

Considering its relevance to other contractile systems, it is clear that whether the phage contractile sheath is a one-component system or two-component system is of considerable importance. This paper is concerned with a direct chemical determination of the number of polypeptide chains in isolated bacteriophage sheaths. Carboxyl end-group analysis by carboxypeptidase treatment and by hydrazinolysis has indicated that there are two different polypeptide chains in T2L contractile sheaths.

MATERIALS AND METHODS

Preparation of T2L phage stocks. Phage was grown, stored, and assayed as described by Kozloff and Lute (13). The osmotic shock method of Herriott and Barlow (11) was used to prepare T2L ghosts. The purification procedure included differential centrifugation in 0.6 M CsCl-deuterium oxide solution. Centrifugation for 75 min at 18,000 × g sedimented all contaminating phage, leaving a supernatant solution containing pure ghosts; this solution was then centrifuged for 2 hr at 40,000 rev/min. The ghost particles were washed twice with 0.9% NaCl plus 10⁻³ M MgSO₄.

Preparation of T2L sheaths. T2L sheaths were prepared by Sarkar, Sarkar, and Kozloff's modification (19) of the acid method (7), with the use of phage ghosts instead of whole phage. For preparation of sheaths for end-group analysis, sulfuric acid buffer at pH 2 rather than glycine-HCl buffer was used to avoid contamination of the sheaths with glycine. After acid treatment, the preparation was freed from contaminating phage head proteins by trypsin and chymotrypsin digestion. The sheath preparations were washed at least three times with 1% ammonium acetate in the preparative ultracentrifuge. The purity of the washed sheath preparations was checked by its sedimentation behavior in a Spinco model E analytical ultracentrifuge. In all cases, only one component with a sedimentation coefficient of 110S was obtained (19).

Further, electron micrographs revealed no other components. As a standard procedure for further purification to obtain sheath preparations of uniform size, the material was run on a 5 to 60% sucrose gradient, and the main peak fractions were pooled. Since concentrated sheath preparations tend to aggregate, on occasion, 1.5 M urea was added before zonal centrifugation. For end-group analysis, the sheath protein preparations were dialyzed extensively against distilled water and then lyophilized.

Hydrazinolysis. Essentially, Bradbury's modification (3, 4) of the method of Akabori et al. (1) was used. A weighed amount of the dry protein, usually 3 to 5 mg, and a known quantity of hydrazine sulfate salt were dried for 3 hr in an ampoule in an evacuated desiccator over sulfuric acid, and then 0.1 ml of anhydrous hydrazine was added to the dried protein salt mixture. The final concentration of the hydrazine salt in the final suspension was 1 M. The ampoule was flushed with nitrogen, sealed under nitrogen, and then heated at 60 C for various periods of time; excess hydrazine was evaporated over sulfuric acid in a vacuum desiccator.

The residue was dissolved in 1 ml of water, 0.4 ml of benzaldehyde was added, and the mixture was neutralized and shaken for 2 hr in the cold. This treatment resulted in the precipitation of any traces of hydrazine remaining as the yellow benzaldehyde complex, while the amino acid hydrazides-benzaldehyde complexes formed a white precipitate. The complexes were sedimented at low speed, and the supernatant fluid containing the free C-terminal amino acids was removed and analyzed in a Beckman amino acid analyzer.

Chemicals used were analytical grade obtained from commercial sources. Anhydrous hydrazine and 1-fluoro-2,4-dinitrobenzene were obtained from Eastman; carboxypeptidase A and B DFP (treated with diisopropylfluorophosphate) were obtained from Sigma Chemical Co., St. Louis, Mo.

RESULTS

N-terminal amino acid analysis. Brenner et al. (7) were unable to detect any N-terminal amino acids in sheath preparations from T2H phage and suggested that the N-terminal group is protected. Our efforts to detect any N-terminal amino groups in T2L sheaths by use of the fluoro-dinitrophenyl method were also unsuccessful. After treatment, sheath preparations did yield significant amounts of ε-DNP lysine, but no detectable amounts of substituted α-amino acids. This confirms the earlier report and very probably indicates the presence of protecting groups on the N-terminal amino acids in the polypeptides comprising the sheath structure. The absence of any α-amino group in sheath preparations supports the view that neither the acid treatment nor the proteases used in preparing sheaths cleave any peptide bonds of the sheath itself.

Carboxypeptidase treatment. Prior dissociation of the sheaths with glacial acetic acid, performed according to the method of Fraenkel-Conrat (10), was found to be unnecessary, since the sheath C-terminal amino acids are readily exposed to enzyme action when suspended in 0.1% sodium dodecyl sulfate. Treatment of the sheath protein with carboxypeptidase B, an exopeptidase specific for basic amino acids, did not release any free amino acids. On the other hand carboxypeptidase A, an exopeptidase, although it does not liberate C-terminal basic amino acids, did liberate the free amino acids shown in Table 1.

Glycine and serine were released in the largest amounts followed by threonine, alanine, and aspartic acid. The glycine-to-serine ratio after incubation for 0.5 hr was almost 2:1, whereas
TABLE 1. Sheath amino acids liberated by carboxypeptidase A

<table>
<thead>
<tr>
<th>Amino acid released</th>
<th>Moles per mole of sheath</th>
<th>Expt I, 0.5 hr at 37 C</th>
<th>Expt II, 12 hr at 37 C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycine</td>
<td>86</td>
<td>230</td>
<td></td>
</tr>
<tr>
<td>Serine</td>
<td>50</td>
<td>280</td>
<td></td>
</tr>
<tr>
<td>Threonine</td>
<td>16</td>
<td>94</td>
<td></td>
</tr>
<tr>
<td>Alanine</td>
<td>14</td>
<td>92</td>
<td></td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>8</td>
<td>76</td>
<td></td>
</tr>
<tr>
<td>Leucine</td>
<td>—</td>
<td>73</td>
<td></td>
</tr>
<tr>
<td>Glutamine</td>
<td>—</td>
<td>65</td>
<td></td>
</tr>
<tr>
<td>Valine</td>
<td>—</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>Tryptophan</td>
<td>—</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>—</td>
<td>71</td>
<td></td>
</tr>
</tbody>
</table>

* The reaction mixture contained sheath protein and enzyme in a ratio of 50:1 by weight. Carboxypeptidase A was washed once with distilled water, dissolved in 2 M \( \text{NH}_{4}\text{HCO}_{3} \); the final concentration of \( \text{NH}_{4}\text{HCO}_{3} \) in the enzyme mixture is 0.2 M. To stop the reaction, an equal volume of 1 N HCl was added, and the sample was centrifuged to remove the residual protein. Supernatant solutions containing the free amino acids were either concentrated for paper chromatography or examined in the amino acid analyzer. Controls using the same conditions in both experiments in the absence of enzyme showed that no free amino acids were present.

after 12 hr of incubation there was more free serine than glycine. Experiment I indicates the very probable presence of glycine C-terminal polypeptides in sheath preparations. The large amount of serine liberated suggests the presence of an additional polypeptide containing C-terminal serine. Of course, it is possible that some or all of the serine may have come from further digestion of the polypeptide with C-terminal glycine.

*Hydrazinolysis.* To determine the total amount of C-terminal amino acids, and thus the number of polypeptide chains per sheath without interference from amino acids further in the sequence, the hydrazine method introduced by Akabori et al. (1) as modified by Bradbury (3, 4) was used. There are numerous difficulties with this procedure for determining carboxyl terminal amino acids quantitatively. As Bradbury (3, 4) and later Braun and Schroeder (6) have pointed out, the kinetics of hydrazinolysis varies greatly with the nature of the specific peptide bond and the specific C-terminal amino acid. Whereas C-terminal glycine is usually released in excellent yields, serine peptides are much more resistant to hydrazinolysis. Braun and Schroeder (6), using a slightly different modification of the original Akabori method, found that prolonged hydrazinolysis of the dipeptide glycyl serine yielded only 34 to 35% of the expected free serine. In our laboratory, hydrazinolysis of the model peptide alanyl serine, even after 24 hr at 60 C, yielded only 60% of the expected serine. In contrast, similar treatment of the model peptide leucyl glycine gave 94% of the expected glycine.

One further difficulty is that amino acid hydrazides tend to hydrolyze spontaneously and thus give high values for end groups. Apparently, this is especially true for glycine hydrazides and to a much lesser extent for the hydrazides of alanine, serine, and the other amino acids. The results obtained by Brown et al. (8) with the hydrazine method suggested that glycine might be C-terminal in \( \beta \)-galactosidase, whereas carboxypeptidase B treatment clearly indicated that lysine was the C-terminal amino acid.

Although Bradbury (4) stated that his hydrazinolysis procedure rarely gives false positive C-terminal amino acids, the limitations of this method clearly bear on the interpretation of the hydrazinolysis experiments given in Table 2. Although the hydrazine method does give somewhat erratic results, these analyses plus those from the carboxypeptidase experiments are enough to indicate the types of C-terminal amino acids and to give some indication of the number of each per sheath particle. In general, glycine and serine were the major amino acids released. In experiments Ia, Ic, and IIc, these were the only free amino acids found. (In experiment Id, where high values were found for alanine, aspartic acid, and threonine, the sample was stored for a prolonged period which permitted considerable hydrolysis.)

| TABLE 2. Sheath amino acids liberated by hydrazinolysis* |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Expt            | Hr at 60 C      | Moles of amino acid per mole of sheath | Glycine | Serine | Alanine | Aspartic acid | Threonine |
| I a             | 8               | 57              | 22              | 0               | 0               | 0               |
| I b             | 16              | 190             | 57              | 31              | 14              | 14              |
| I c             | 24              | 210             | 52              | 0               | 0               | 0               |
| I d             | 48              | 176             | 45              | 124             | 53              | 8               |
| II a            | 1               | 31              | 13              | 4               | 2               | 0               |
| II b            | 2               | 20              | 14              | 5               | 0               | 0               |
| II c            | 4               | 75              | 33              | 0               | 0               | 0               |
| III a           | 3               | 200             | 70              | —               | —               | —               |

* Experiment I was carried out on sheath preparation 1 and experiments II and III were on preparation 2.
In spite of the limitations of each individual hydrazinolysis experiment, the kinetics of release of both glycine and serine given in Fig. 1 support the conclusion that there are at least two polypeptide chains in sheaths. Since both curves go through the origin, glycine and serine must be released simultaneously rather than sequentially. The difference in rate of release is most likely due to the slower hydrazinolysis of peptidic bonds containing serine.

**Correction of experimental C-terminal glycine and serine values.** End-group analysis can only give a minimal number of the types of polypeptide chains, but, with the assumption that there are only two types, an estimate can be made of the relative amount of each. Around 200 moles of glycine are found per mole of sheath. This value is probably too high based on the known lability of glycine hydrazides. Bradbury (4) reported that treatment of insulin resulted in 8 µmoles of non-terminal glycine per g of protein, whereas lysozyme yielded 5 µmoles of nonterminal glycine per g of protein. Brown et al. (8) found about 3 µmoles of non-terminal glycine per g of β-galactosidase. It is apparent that a correction must be made for the non-terminal glycine released. If an average of 5 µmoles of glycine per g of protein is produced, then Bradbury's hydrazinolysis method would yield 40 extra moles (5 µmoles × 8 × 10^6) of non-C-terminal glycine per mole of sheath. A similar estimate for this correction can be made using the value given by Akabori et al. (1) of 0.6% for the liberation of free glycine from glycine hydrazides. Since sheaths contain 5,500 total glycine residues (19), 30 to 40 extra non-terminal glycines would be found. When a correction of 40 moles of glycine is subtracted from the value obtained of 194 ± 14 moles per mole of sheath, the number of C-terminal glycine residues is reduced to about 154 (±30) moles per mole of sheath.

Corrections also have to be considered for the C-terminal serine value, although in this case the C-terminal serine value is most likely too low. Hydrazinolysis of the model peptide alanyl-serine in this laboratory gave only 59% of the expected serine, which would mean a correction factor of 1.7. Braun and Schroeder's (6) studies on the model peptide glycy1-serine gave an even larger correction factor of about 2.9. The residue adjacent to the C-terminal serine in sheaths is unknown, and an average correction factor of about 2.3 ± 0.4 seems reasonable. Further, this is close to the ratio of the relative rates of release of the two amino acids given in Fig. 1. Since the average of the C-terminal serines found was 56 ± 10 (from experiments Ib, Ic, Id, and IIIa), the corrected number of moles of C-terminal serine per mole of sheath would be about 130 (±45).

Although the uncorrected data give a C-terminal glycine to serine ratio of between 3:1 and 2:1, the corrected values support the view that there are about the same number of C-terminal serine residues as there are C-terminal glycine residues. Further, in view of Moody's (16, 17) electron microscopic evidence for the presence of 144 visible "large" and "small" subunits, it seems quite likely that one of them has a C-terminal glycine and the other a C-terminal serine. This conclusion would be greatly strengthened by isolating these components, but all efforts to isolate any subunits from sheaths by centrifugation, electrophoresis, or chromatography, even in the presence of reagents such as 10 M urea, 2 M guanidine, and strong acids or bases, have been unsuccessful.

**Discussion**

The normal extended tail sheaths of various T-even bacteriophages have their subunits arranged in 24 annuli; upon contraction, there are only 12 annuli (7). Moody (16, 17) and Krimm and Anderson (15) agree that the conformational change involves a merging of pairs of annuli, together with some "right handed" rotation of the sheath subunit components around the main axis of the sheath. These observations eliminate the possibility that all of the sheath components are arranged helically and that contraction in-
volves only a change in the pitch of the helix (18). This concept of merging of annuli involves a change in the geometry of the interaction of neighboring sheath components and most likely a change in the conformation of the sheath components themselves. This last observation is derived from the change in optical rotary dispersion discussed by Polglasov (18).

The current finding that there are two kinds of polypeptide chains, possibly in equal numbers, in sheaths is not in conflict with these physical observations or with these models of the conformational changes. The presence of two types of polypeptide chains does point to the problem of the nature of the bonds between sheath components in one annuli and the bonds between annuli. It seems possible that one of the critical events during contraction is a change in the bonds between a "large" subunit in one annulus with a "small" subunit in an adjacent annulus. Two types of interactions between different polypeptide chains would appear to offer more opportunity for the conformational change observed than would a major refolding of a single polypeptide chain.

So far, only three phage genes, 3, 15, and 18, have been found necessary for the construction of the final phage sheath (Edgar, Lielausis, and King, personal communication). The gene 18 product appears to be a major polypeptide component of the sheath, since the action of this gene allows the formation of an unstable sheath structure about the tail tube. The products of genes 3 and 15 are necessary to stabilize the sheath structure. Based on the evidence in this report for two polypeptide chains, it seems possible that either gene 3 or 15 may be responsible for the second polypeptide component.

ACKNOWLEDGMENT

This investigation was supported by Public Health Service grant AI 06336 from the National Institute of Allergy and Infectious Diseases.

LITERATURE CITED