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LPP-1 Infection of the Blue-Green Alga *Plectonema boryanum*

III. Protein Synthesis

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The structural proteins of mature LPP-1 particles and the patterns of protein synthesis after LPP-1 infection have been examined by electrophoresis on sodium dodecyl sulfate polyacrylamide gels. Structural proteins account for 35% of the LPP-1 genome, and proteins that would require about 65% of the total coding capacity have been detected after infection. The major head proteins have molecular weights of 39,000 and 13,000, whereas the major tail protein is an 80,000-molecular-weight species. Host protein synthesis is depressed soon after infection and appears to be entirely shut off by 5 hr. Three classes of viral proteins are distinguished in infected cells, based on their time course of synthesis and their presence in mature virions.

Electrophoresis on acrylamide gels provides sufficient resolution to analyze the program of protein synthesis in viral infection in considerable detail (14). In the preceding paper we noted several similarities in deoxyribonucleic acid (DNA) metabolism in LPP-1-infected *Plectonema boryanum* and in T7-infected *Escherichia coli*. We characterized the structural proteins of LPP-1 and followed the time course of protein synthesis during LPP-1 infection by electrophoresis on acrylamide gels in buffers containing sodium dodecyl sulfate (SDS) (7, 8). Again, the similarity to T7 and T7 infection is striking. LPP-1 contains at least 10 structural proteins, and three classes of proteins can be distinguished during LPP-1 infection.

MATERIALS AND METHODS

Purification of virus and viral components. Algae and virus were grown as described in an accompanying paper (11). For the electrophoresis of viral components, the virus was first purified twice on CsCl step gradients (11). The major virus-containing fractions were pooled and dialyzed against 10^{-2} M $MgCl_2$ in 0.01 M tris(hydroxymethyl)aminomethane, *p*H 7.5 (TM buffer). Viral heads and tails were separated by the method of Luftig and Haselkorn (6), except where noted.

Polyacrylamide gel electrophoresis. Labeled, purified virus was made 1% in SDS and β-mercaptoethanol, boiled for 1 to 5 min, and dialyzed overnight at 20 C against 100 volumes of 0.1% SDS, 0.1% β-mercaptoethanol in 0.01 M PO_4, *p*H 7.2 (PSM) (7, 8). Protein synthesis in infected cells was followed by adding $^{35}SO_4^{=}$ (ICN) at 1 to 100 μCi/ml and a specific activity of 100 to 1,000 mCi/mmole to an infected culture for the prescribed time, adding $CHCl_3$, and chilling. The cells were spun down, washed twice, and resuspended in Chu medium. The cells were then lysed by treatment with 100 μg of lysozyme per ml in 0.1 M ethylenediaminetetraacetic acid (EDTA) for 4 hr at room temperature (or 12 hr at 4 C), sonically treated for 10 min at setting 6 in a Bronson Sonifier, and treated with 10 μg of deoxyribonuclease per ml. Treatment for shorter periods of time with lysozyme (1 hr at 37 C) gave identical gel patterns but resulted in incomplete cell breakage. The cells were not all solubilized in SDS; longer development times were necessary, and a larger percentage of the radioactivity remained at the top of the gel. The extract was then made 1% in SDS and β-mercaptoethanol, heated at 100 C for 1 to 5 min, and then dialyzed overnight against PSM. About 100,000 to 300,000 dpm were layered on each gel.

For molecular weight determinations, known protein standards were run either in the same gels with the labeled extracts or separately (1, 9, 10). The two methods gave identical results. The standards used were: chymotrypsinogen, malate dehydrogenase, and phosphorylase a (Worthington Biochemical Corp.); α-amylase (Sigma Chemical Co.); myoglobin and cytochrome *c* (Calbiochem); catalase (Boehringer). The molecular weights used were taken from Klotz and Darnall (4).

After electrophoresis, gels were fixed overnight in 20% sulfosalicylic acid, stained for 2 to 3 hr in 0.25% Coomassie brilliant blue (Colab) in water–acetic acid–methanol (5:1:5), and destained in a 7.5%

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acetic acid–5% methanol solution by diffusion. Eliminating the staining yielded identical results. The gels were sliced and autoradiographed by the method of Fairbanks (2). The autoradiograms were traced on a Joyce-Loebl densitometer. Relative proportions were determined by cutting out the peaks and weighing.

RESULTS

Labeling the proteins of blue-green algae is complicated by the poor incorporation of ^{14}C-labeled organic compounds (13) by these organisms. This difficulty was overcome by using carrier-free $^{35}SO_4^{=}$. By resuspending logarithmically growing *P. boryanum* in fresh medium 12 hr before the addition of label, incorporation proceeded without a noticeable lag and remained linear until the label was exhausted or until lysis.

The specificity of this incorporation was tested by a chase with 1% Casamino Acids and 0.1 M $MgSO_4$. This depressed incorporation immediately, but not completely. The extent of incorporation of $^{35}SO_4^{=}$ into acid-precipitable material in the presence of 1% Casamino Acids was approximately 10% of the incorporation in controls.

It was then necessary to determine whether this residual incorporation was into protein or some other substance. To do this, cells were labeled for three generations with $^{35}SO_4^{=}$, opened with lysozyme, sonically treated, and incubated in the presence of Pronase. After 72 hr of Pronase treatment, 9% of the radioactivity remained acid-precipitable. This strongly suggests that approximately 10% of the $^{35}SO_4^{=}$ incorporated enters a nonprotein component, possibly a sulfated polysaccharide.

This supposition is lent further strength by measuring the inhibition of $^{35}SO_4^{=}$ incorporation with chloramphenicol (CAP). This antibiotic is known to stop protein synthesis in organisms containing 70*S* ribosomes (e.g. 17). Since blue-green algae contain 70*S* ribosomes, CAP should block protein synthesis and, therefore, $^{35}SO_4^{=}$ incorporation. The results shown in Table 1 were obtained when we measured $^{35}SO_4^{=}$ incorporation in the presence of various concentrations of inhibitor. At concentrations of 50 and 100 μg/ml, over 90% inhibition of $^{35}SO_4^{=}$ incorporation is obtained. Since the 8% of $^{35}SO_4^{=}$ incorporation that is resistant to CAP does not exceed the proportion of radioactive product insensitive to Pronase, we conclude that CAP completely inhibits protein synthesis in *P. boryanum*. Moreover, at least 90% of the radioactivity distributed in bands on the gels to be shown is in protein. CAP at 100 μg/ml also completely blocked the synthesis of virus.

To observe clearly the early synthesis of viral proteins, we attempted to depress the synthesis of host proteins by irradiation with ultraviolet light. This technique was used successfully in T4 and T7 infection of *E. coli* (3, 14). In those cases, host protein synthesis was completely eliminated, although phage proteins were made in reasonable quantities. This proved to be imposible in LPP-1 infection of *P. boryanum*. In Table 2, we show that, at a dose which reduced viral yield over 100-fold, $^{35}SO_4^{=}$ incorporation into host protein is only reduced four- or fivefold. This result was obtained by using an intensity of 72,000 ergs/mm², which is 10 times the dose used by Studier and Maizel on *E. coli* (14). Therefore, we could not depress the synthesis of host proteins, and these will be seen early in infection in the autoradiograms to be presented.

TABLE 1. *Effect of chloramphenicol (CAP) on $^{35}SO_4^{=}$-incorporation in Plectonema boryanum*[a]

CAP concn (μg/ml)	Inhibition of incorporation (%)
1	61
10	81
50	91
100	92

[a] *P. boryanum* cells were collected by centrifugation and resuspended in fresh medium 12 hr before the addition of 1 μCi of $^{35}SO_4^{=}$ per ml. CAP was added 1 hr later and incorporation was followed for 24 hr; 0% inhibition = 38,000 counts per min per ml.

Protein composition of mature LPP-1 particles. Ten bands can be distinguished on electrophorograms of purified LPP-1 disrupted by boiling in SDS-mercaptoethanol. A densitometer tracing of an autoradiogram of ^{35}S-labeled LPP-1 proteins is shown in Fig. 1. The two major bands, 8 and 13, contain over 70% of the total radioactivity. The molecular weight of each band was determined by coelectrophoresis with a set of standards, with the results shown in Table 3. The table also includes a tentative assignment of bands to structural components of the virus, obtained in the following way. A solution of CsCl-purified virus was adjusted to 0.1 M in EDTA and left at room temperature for 30 min. This step dissociates LPP-1 into heads and tails (6). $MgCl_2$ and deoxyribonuclease were added, and, after a period for digestion of DNA and dialysis against 10^{-3}M EDTA, heads were separated from other proteins by pelleting through a 5 to 20% sucrose gradient for 30 min at 50,000 rev/min. To the supernatant, a two-drop portion of concentrated LPP-1 antiserum (K $\sim$ 600) was added which caused a flocculent precipitate to form. This precipitate was collected by centrifugation for

15 min at 50,000 rev/min and was considered to be the tail fraction. Protein remaining in the antiserum supernatant was considered to be internal protein.

TABLE 2. *Effect of ultraviolet (UV) irradiation on incorporation of* $^{35}SO_4$ *into Plectonema boryanum and on the growth of LPP-1*[a]

UV dose	$^{35}SO_4$ counts per min incorporated per hr per ml		Proportion of infectious centers yielding virus	Burst size
	Uninfected	LPP-1-infected		
None	2,100	1,800	1.0	5,000
5 min (36,000 ergs/mm²)	600	200	0.14	200
10 min (72,000 ergs/mm²)	400	200	0.06	500

[a] *P. boryanum* cells were irradiated 0.5 hr before infection, kept in the dark until infection (MOI = 10), and left in the dark for 2 more hr. At 2 hr, 1 μCi of $^{35}SO_4$ per ml was added to part of the culture, and dilutions were made for one-step growth analysis.

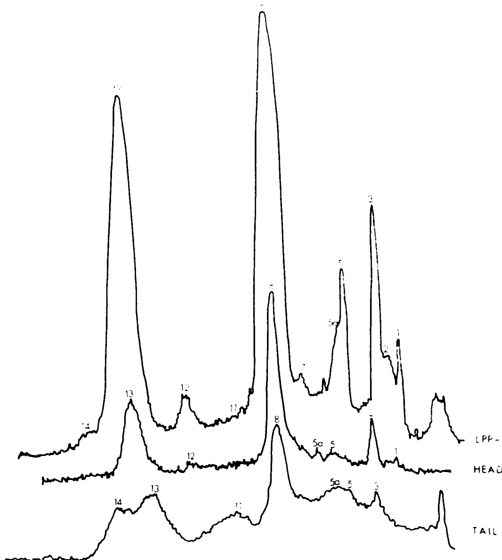

FIG. 1. *Electrophoretic separation of LPP-1 proteins on SDS-acrylamide gels. Heads and tails were fractionated as described in the text. Electrophoresis on 7.5% gels was from right to left. Fixing, slicing, radioautography of* ^{35}S, *and densitometry were as described in Materials and Methods. The numbering system is based on all of the proteins found in LPP-1-infected cells. Molecular weights of the proteins are shown in Table 3.*

TABLE 3. *Structural proteins of LPP-1 and the relative distribution of label in the major components*

Band no.	Molecular weight	Structure	Label in component
			%
1	95,000	Head	
2	87,000	Head	
3	80,000	Tail	8.0
5	62,000	Tail	7.4 (5 and 5a)
5a	60,000	Tail (?)	
8	39,000	Head (major)	36.0
11	26,000	Tail	
12	19,000	Head	
13	13,000	Head (major); tail	39.0
14	10,000	Tail	

Densitometer scans of the autoradiograms of isolated heads and tails are shown in Fig. 1. Band 8, which comprises over a third of the total label, appears to be the major head structural protein. Band 13 also is an important head constituent. The molecular weights of these proteins are 39,000 and 13,000, respectively.

The tail fraction of LPP-1 (Fig. 1) is interesting since there are many bands that correspond to head proteins. Electron microscopic examination of these fractions showed little intact head contamination, so that, if these proteins are of head origin, they must be from heads that have fallen apart during EDTA treatment. This also implies that the LPP-1 antiserum is active against head proteins as well as against the tail antigens. The only proteins found in the tail fraction that are not found in the head fraction are proteins 3, 11, and 14. From the relative proportions of bands 8 and 13, it appears that band 13 may be a tail component as well as a head component. Protein 3 is most probably the major tail component, considering its abundance in whole virus. The major head component is also the most abundant protein in the antiserum-precipitated fraction, presumed to be tail proteins. Some of this head protein, as mentioned previously, may come from completely dissociated heads. In addition, some head subunits may be firmly associated with the tail, particularly in the structure called the tail capital by Luftig and Haselkern (6). All of the proteins found in the whole virus appear in the head or tail fractions or in both, and no radioactivity was recovered in the antiserum supernatant of LPP-1. Either there are no internal proteins in LPP-1, or they contain no sulfur.

Time course of protein synthesis after LPP-1 infection. The time course of protein synthesis after LPP-1 infection was analyzed by pulse-label-

ing infected cells [multiplicity of infection (MOI) =10] with ^{35}S for 1 hr and electrophoresing the boiled extracts on 7.5% SDS polyacrylamide gels. This solubilization procedure gave reproducible results with 95% of the label entering the gel after 7 hr of electrophoresis at 6 ma/gel. The patterns obtained are shown in Fig. 2. The labeling pattern shows synthesis of proteins hour-by-hour, from which we can compile a scheme for post-infection protein synthesis shown in Fig. 3. The disappearance of a band means that its synthesis is shut off or depressed below the resolution of the autoradiographic technique. Appearance of a new band means that its synthesis is in sufficient quantity to be detected on the gels. The scheme, then, is approximate, and the timing should not be considered exact.

The patterns of the first 5 hr are radically different from those of the last 6 hr. The host proteins that dominate the early patterns are slowly shut off, and by 5 hr host protein synthesis appears to have stopped entirely. It is not possible to decide if the low molecular weight (≤12,000) proteins belong to the host or to the virus. These labeled extracts were electrophoresed on 10% gels as well to attempt a better resolution in this region, but there were always at least two diffuse bands present. However, from these patterns it is possible to conclude that at least most of the host protein synthesis is shut off within the first 5 hr after infection.

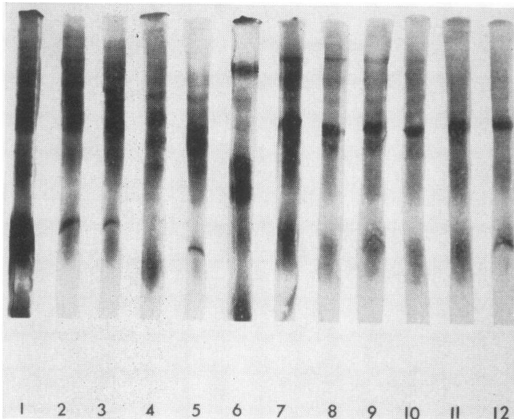

FIG. 2. *Proteins synthesized during LPP-1 infection. P. boryanum cells were infected with LPP-1 at MOI = 10, pulsed for 1-hr periods with 100 μCi of ^{35}S per ml and prepared for electrophoresis as described in Materials and Methods. Electrophoresis was for 7 hr at 6 ma/gel, and the direction of migration was from top to bottom. The autoradiograms were exposed for 10 days. The dye markers ran about 7 cm except for no. 6 which ran about 9 cm. Pulse time of the gels was as follows: gel no. 1, uninfected cells, 0 to 10 hr; 2, infected cells, 0 to 1 hr; 3, infected cells, 1 to 2 hr; 4, infected cells, 2 to 3 hr; 5, infected cells, 3 to 4 hr; 6, infected cells, 4 to 5 hr; 7, infected cells, 5 to 6 hr; 8, infected cells, 6 to 7 hr; 9, infected cells, 7 to 8 hr; 10, infected cells, 8 to 9 hr; 11, infected cells, 9 to 10 hr; and 12, infected cells, 10 to 11 hr.*

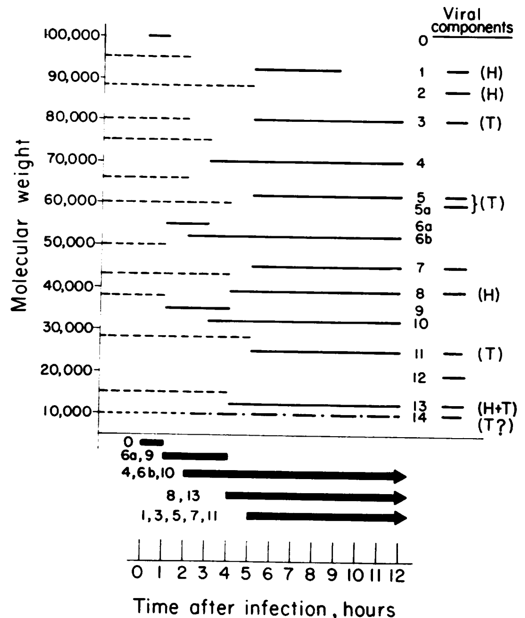

FIG. 3. *Scheme for protein synthesis after infection of P. boryanum with LPP-1 (MOI = 10). Dashed line indicates host proteins; solid line represents viral proteins. Viral-coded proteins are numbered from 0 to 14 on the right-hand side. Those proteins also found in mature virus particles are designated by horizontal bars, and their location in heads or tails is indicated. The timing of synthesis of viral-directed proteins is summarized in the bottom part of the figure. Bands 0, 6a, and 9 belong to the earliest class of proteins; bands 4, 6b, and 10 belong to the early class; and bands 1, 3, 5, 7, 8, 11, and 13 comprise the late class.*

In T7 infection of *E. coli*, the viral proteins synthesized after infection have been divided into three classes: earliest, early, and late (14). We can make a similar assignment of the proteins in LPP-1 infection (Fig. 3). The earliest class is defined as containing those proteins synthesized soon after infection which are also shut off by 4 hr. This class consists of three proteins of 100,000, 55,000, and 35,000 molecular weight. The early class consists of proteins whose synthesis begins around 2 hr and continues until lysis but are not found in mature virus particles. This class contains three proteins also (70,000, 52,000, 32,000). Finally, late proteins are viral structural proteins whose synthesis begins at about 4 hr and con-

tinues until lysis and can be found in isolated viral particles.

In the patterns obtained from labeled cell extracts, a total of 17 distinct viral-directed proteins have been detected. They represent a combined molecular weight of near 900,000 daltons, which accounts for about 65% of the coding capacity of the viral genome. Of the remainder, some may be enzymes that are present in catalytic amounts and that would not be present in high enough quantity to be detected. Others may be necessary for assembly of mature virions but present in quantities below the resolution limit.

We have tried to determine whether certain of the LPP-1 structural proteins are initially synthesized as large polypeptides which are later cleaved to yield the final proteins of the mature virus. This precursor-product relationship has been shown to occur during poliovirus infection (15) and for certain T4 proteins (5). In a number of experiments, the cells were pulsed from 5 to 5.25 hr and from 6 to 6.25 hr and then chased with 1% CAA and 0.1 M $MgSO_4$ for 2 to 3 hr which effectively inhibits ^{35}S-incorporation by 90%. These chased extracts were run on 7.5 and 10% gels; however, no redistribution of label occurred during the chase. This would imply that no large precursors are synthesized during LPP-1 infection, which is again similar to the case with T7 (14).

DISCUSSION

We have been able to characterize the major structural proteins of LPP-1 by using SDS gel electrophoresis. These proteins comprise a total molecular weight of almost 500,000 daltons, which would require about 35% of the coding capacity of LPP-1 DNA. The major head proteins are the 39,000- and 13,000-molecular weight species. This is substantially different from the 17,500-molecular weight protein found by Luftig and Haselkorn (6) to be the only protein in the LPP-1 capsid. However, the urea treatment they used could easily disrupt the proteins to give only one species. For example, if urea solubilized protein 13 but left protein 8 aggregated, only the former would have entered the urea-Sephadex column of Luftig and Haselkorn. The results of SDS electrophoresis on other viruses are comparable to ours. For T7, Studier and Maizel (14) found a molecular weight of 35,000 for the major head protein. We found no evidence for the synthesis of large precursors for any structural protein during LPP-1 infection.

After infection with LPP-1, host protein synthesis is shut off by 5 hr, and at least three different classes of viral proteins begin to be synthesized. The identification of three classes is tentative—it is possible that proteins below the resolution of the present technique will be found that can alter this scheme somewhat.

A number of the bands obtained from gels of infected lysates can be identified as viral structural proteins (Fig. 1). It would be of interest to identify other bands with specific functions in viral development. We know that proteins required for host DNA breakdown and for viral DNA synthesis are synthesized during the early stages of viral infection (12); it is thought that these are members of the earliest and early classes, respectively. However, it will not be possible to identify many functions until conditional lethal mutants are obtained.

Since many other analogies between LPP-1 and T7 infection have been mentioned so far, we would like to make one further comparison. The gene 1 product of T7 has been shown to be a viral-directed transcription factor (molecular weight = 100,000) that is required for transcription of early and late T7 messenger ribonucleic acid (16). This gene product is synthesized very early after T7 infection and has been placed in the earliest class. The first viral protein synthesized after infection with LPP-1 has a molecular weight of 100,000 and is labeled in detectable amounts only during the 1st hr. It is tempting then to suggest that this is a viral-directed transcription factor and that a transcription control mechanism similar to that of T7 exists for LPP-1.

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