Replication of Bacteriophage SH-133 in the Facultative Autotroph *Hydrogenomonas facilis*

II. Bacteriophage Synthesis and Hydrogenase Induction Under Step-Up and Step-Down Growth Conditions

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Autotrophically grown infected cells are able to replicate phage SH-133 after being switched to a heterotrophic environment (step-up growth). The effect of step-down growth on phage replication varies with the choice of organic substrate. Phage replication and the induction of cellular hydrogenase occur under step-down growth from acetate but not peptone broth. The requirement of a continued source of energy for phage replication in either heterotrophically or autotrophically grown cells could be uniquely demonstrated in this phage-host system by the deletion of hydrogen from the growth medium.

Hydrogenomonas facilis is a facultative autotroph, able to grow heterotrophically with an organic carbon source or in an inorganic salts medium with sodium acetate as a carbon and energy source. Alternatively, *H. facilis* will grow autotrophically in an inorganic salts medium if a mixture of H₂, CO₂, and air is supplied; hydrogen is utilized as the energy source and CO₂ is utilized as the carbon source (4, 9).

The utilization of molecular hydrogen requires the induction of a hydrogen oxidizing system (7, 11). The chemical energy released is then converted to biologically utilizable energy in the form of ATP through oxidative phosphorylation (T. Soulen, Dissertation Abst. 24:2256-2257).

H. facilis exhibits some hydrogenase activity when grown under air on a variety of organic media, although at values much less than that determined for autotrophically grown cells (1, 2, 6, 9). Lindsay and Syrett (7) found that *H. facilis* had no detectable hydrogenase activity when grown heterotrophically under air, but activity appeared when the culture was incubated in a mixture of hydrogen and oxygen. This suggests the induced synthesis of hydrogenase in the presence of hydrogen.

H. facilis can simultaneously oxidize molecular hydrogen and an organic substrate (3, 7, 11), and the activity of the hydrogen oxidizing system is not dependent upon the presence of carbon dioxide (7).

We already investigated the effect of SH-133 phage infection on the macromolecular synthesis of the host cell. In either autotrophically or

heterotrophically (peptone) grown infected cells, there is an immediate cessation of host macromolecular synthesis. However, under heterotrophic growth with acetate, host synthesis continues to function throughout the greater part of the latent period (12). These observations prompted an investigation of the functional role of the host cell in the synthesis of SH-133. This raised the following questions: (i) what is the effect on phage replication of a change in the carbon or energy source after infection, and (ii) what is the effect of phage infection on the induction of cellular hydrogenase, an enzyme essential for autotrophic metabolism.

The present paper investigates the effect of phage infection under step-up and step-down growth conditions on the production of infectious phage particles and the induction of cellular hydrogenase.

MATERIALS AND METHODS

Bacterial strains and bacteriophage. The original strain of *H. facilis* ATCC 15376 was obtained from R. Burris, Department of Biochemistry, Univ. of Wisconsin. This strain was used in all studies. Phage strain SH-133 was isolated in this laboratory.

Growth conditions. The defined basal medium (Ks) had the following composition: KH₂PO₄, 2.0 g; Na₂HPO₄, 6.0 g; NH₄NO₃, 1.0 g; MgSO₄, 0.15 g; and distilled water, 1.0 liter. For autotrophic growth, a gas mixture of 10% CO₂, 60% H₂, and 30% air (autotrophic atmosphere) was supplied; for heterotrophic growth, 1.2 mg of sodium acetate per ml was added.

The complex medium peptone broth, 4 g of peptone

per liter of distilled water, was used for liquid cultures. All phage assays were done in peptone broth supplemented with agar.

Step-up growth conditions. Cells were grown to a concentration of 10^6 cells per ml in Ks medium with an atmosphere of CO_2 , H_2 , and air. The cells were centrifuged down and resuspended in fresh Ks medium containing 1.2 mg of sodium acetate per ml or in 0.4% peptone broth.

For phage replication, the resuspended cells were infected with phage, and, after a 10-min adsorption period, were centrifuged, and resuspended in Ks medium or peptone broth.

Step-down growth conditions. Cells were grown in medium Ks with limiting organic substrate (200 μg of sodium acetate per ml or 250 μg of peptone per ml). Growth was followed turbidimetrically, and when the substrate was exhausted the cells were centrifuged and resuspended in Ks medium and provided with CO_2 , H_2 , and air.

For phage replication the resuspended cells were infected with phage, allowed a 10-min adsorption period, recentrifuged, and resuspended in Ks medium with the proper gas mixture. All experiments were done at 29 C. Liquid cultures were incubated on a New Brunswick water-bath rotary shaker.

Hydrogenase assay. Samples were frozen in a dry ice-alcohol mixture. The cells were thawed, centrifuged down, and washed twice in 0.07 M Sorensen phosphate buffer (pH 7.2), with the protein concentration adjusted to 0.5 mg/ml. Hydrogenase activity was measured by adding 1 ml of the cell suspension to the side arm of a Thunberg tube. Four milliliters of phosphate buffer (pH 7.2) and 1 ml of 0.12 mM methylene blue were added to the main tube. The tube was evacuated, and the air was replaced with hydrogen for the experimental sample or nitrogen for the control. The contents were mixed, and the decrease in adsorption was measured at 660 nm (5).

RESULTS

The effect of step-up and step-down growth conditions on the growth of *H. facilis* is shown in Fig. 1. Under step-up growth conditions, addition of peptone to autotrophically growing cells resulted in an immediate increase in cell growth. However, under step-down culture conditions, addition of CO_2 , H_2 , and air after exhaustion of limited concentrations of acetate or peptone resulted in a lag period before the onset of autotrophic growth. If host cell functions are essential for phage replication, under step-down growth conditions no phage should be synthesized, whereas autotrophically grown infected cells should support phage replication after the addition of organic substrates (step-up growth). Decrease in optical density resulting from the lysis of the cells was used as an indication of phage replication.

The effect of phage replication on cell turbidity under step-up growth conditions, when autotrophically grown cells were infected and placed in either Ks medium containing sodium acetate

FIG. 1. Growth of *Hydrogenomonas facilis* under step-up and step-down growth conditions. Step-down growth (O): cells were cultured on limiting organic substrate (Ks medium plus 200 μg of acetate per ml or 250 μg of peptone per ml). Step-up growth (●): cells were cultured autotrophically and switched to Ks medium containing either acetate or peptone. Symbols □, and ■ are heterotrophic and autotrophic controls, respectively. Arrows A and B indicate time of addition of either autotrophic atmosphere or organic substrate, respectively.

or peptone broth, is shown in Fig. 2. The average burst sizes under step-up growth conditions, with either acetate or peptone, are similar to those burst sizes determined in either acetate or peptone grown cells, i.e., 8 and 26, respectively.

Under step-down growth conditions, when heterotrophically grown cells were infected and placed in Ks medium under an autotrophic atmosphere, cell lysis did not occur with peptone-grown cells but did occur with acetate grown cells (Fig. 3). Peptone-grown cells showed a 97% loss of PFU, whereas there was no loss of PFU with acetate grown cells (Table 1). The average burst size under step-down growth conditions with acetate-grown cells is the same as with autotrophically grown cells. Nitrogen was substituted for H_2 or CO_2 in autotrophically grown infected cells to determine the depend-

FIG. 2. Lysis of autotrophically infected *Hydrogenomonas facilis* under step-up growth conditions. O, Cells cultured under autotrophic atmosphere. Arrows A, B, and C indicate time of atmosphere removal, infection, and addition of organic substrate, respectively. Experimental: addition of phage plus peptone (▲), addition of phage plus acetate (Δ). Controls: addition of phage plus atmosphere (□), addition of atmosphere minus phage (●), and addition of phage minus organic substrate (■).

ence of phage replication on the organic substrate during step-up growth as either an energy source or as a carbon source, or both. No increase in phage after incubation in either the absence of a carbon (CO_2) or energy (H_2) source demonstrated that the organic substrate is required to fulfill both functions during infection under step-up growth.

The ability of heterotrophically grown infected cells to synthesize hydrogenase for the utilization of hydrogen as an energy source was examined. The hydrogenase activity in acetate-grown cells 20 min after infection was approximately the same as the level found in autotrophically grown cells (94%) (Table 2). The preinfection level of hydrogenase activity was low, which shows that hydrogenase activity was induced postinfection. Induction of hydrogenase activity did not occur in peptone-grown infected cells.

To determine whether the energy level present in acetate-grown cells was sufficient for phage replication under step-down growth, acetate-grown cells were infected and placed under an atmosphere of 60% N_2 + 10% CO_2 + 30% air. No increase in phage after a 3-h incubation period indicated that the infected cell must utilize hydrogen for energy.

DISCUSSION

Change from autotrophic growth conditions to heterotrophic resulted in an immediate increase in cell growth, which shows that enzymes functional in heterotrophic metabolism are con-

stitutive in the autotrophically grown cell. The reverse was not true; heterotrophically grown cells contained low or nondetectable levels of the enzyme hydrogenase which is required for the utilization of hydrogen as an energy source. A lag period occurred in cell growth under step-down growth conditions before the appearance of hydrogenase (Fig. 1).

Under step-up growth conditions, the ability of autotrophically grown cells to synthesize phage after being switched to a heterotrophic

FIG. 3. Lysis of heterotrophically infected *Hydrogenomonas facilis* under step-down growth conditions. Cells cultured on limiting organic substrate (O) of either 200 μg of acetate per ml or 250 μg of peptone per ml. Arrows A, B, and C indicate time of substrate exhaustion, infection, and addition of autotrophic atmosphere, respectively. Experimental: addition of phage plus autotrophic atmosphere to acetate grown cells (□), addition of phage plus autotrophic atmosphere to peptone-grown cells (■). Controls: addition of acetate minus phage (●), addition of peptone minus phage (○), addition of autotrophic atmosphere to peptone grown cells 20 min prior to infection (Δ), and addition of phage minus autotrophic atmosphere (▲).

TABLE 1. Plaque-forming ability of heterotrophically grown infected cells under step-down growth conditions

Substrate		PFU/ml	Autotrophic atmosphere added 20 min before infection
Initial	Final		
Peptone	Autotrophic	1.7×10^4	8.7×10^5
	Peptone ^a	9.4×10^5	
Acetate	Autotrophic	9.2×10^5	9.0×10^5
	Acetate ^a	9.1×10^5	

^a Control.

TABLE 2. *Hydrogenase activity in infected and uninfected cells under step-down growth conditions*

Conditions	Δ OD ^a (N ₂)	Δ OD (H ₂)	Δ OD (H ₂ -N ₂)	Sp act ^b of hydrogenase	Percentage autotrophic
Autotrophic	0.60	1.72 ^c	1.42	5.68	100
Heterotrophic					
Peptone	0.65	0.64 ^a	0.01	0.02	<0.01
Acetate	0.58	0.62 ^a	0.06	0.12	0.02
Peptone step-down					
Uninfected ^d	0.60	1.53 ^c	1.23	4.92	87
Infected ^e	0.63	0.60 ^a	<0.01	<0.01	<0.01
Acetate step-down					
Uninfected ^d	0.63	1.54 ^c	1.73	4.92	87
Infected ^e	0.55	1.60 ^c	1.33	5.32	94

^a 500 milligrams of protein per milliliter.^b Δ Optical density (OD) per milligram of protein per minute.^c 250 milligrams of protein per milliliter.^d 20 min after addition of autotrophic atmosphere.^e Phage added 10 min before addition of autotrophic atmosphere.

environment supports the view that autotrophs possess essentially the same enzyme complement as do heterotrophs (8). The possibility that enzymes necessary for heterotrophic metabolism are being synthesized de novo after infection seems remote since infection of autotrophically grown cells results in an immediate cessation of host macromolecular synthesis (12). In addition, depriving autotrophically grown infected cells of either hydrogen or carbon dioxide, or both, results in an inability to synthesize phage; thus, phage synthesis under step-down growth is due neither to the pre-existing energy level of the host cell nor to the recycling of host proteins into phage proteins.

The effects of step-down growth on phage replication were found to vary with the choice of organic substrate. Step-down growth from peptone broth resulted in a 97% loss in PFU, but no loss when the cells were pregrown with acetate. Either acetate-grown cells, unlike peptone-grown cells, contain the enzymes necessary for growth in an autotrophic environment, or enzymes necessary for autotrophic growth are induced in acetate-grown cells after phage infection. Synthesis of RNA and DNA continues after infection in acetate-grown cells, but not in peptone-grown cells (12). The induction of a specific enzyme essential for autotrophic growth, hydrogenase, which is not present in the heterotrophically grown cell, is possible in the acetate-but not the peptone-grown infected cell. Hydrogen added 20 min before the infection of peptone-grown cells resulted in phage rescue under step-down growth, suggesting

that the induction of hydrogenase is occurring after phage infection in acetate-grown infected cells under step-down growth. Both the induction of hydrogenase and the demonstration of its functional role in phage synthesis under step-down growth indicates that host cell enzymes are essential for SH-133 phage replication. The possibility that acetate-grown cells contain energy levels sufficient for phage replication before infection has been ruled out.

The requirement that the continued synthesis of energy is needed for phage replication in either heterotrophically or autotrophically grown cells can be uniquely demonstrated in this phage-host system by the deletion of hydrogen from the environment and should prove to be a useful model system for the further demonstration of phage-host cell interactions.

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