STIMULATION OF β-GALACTOSIDE TRANSPORT IN *PSEUDOMONAS* BAL-31 BY INFECTION WITH BACTERIOPHAGE PM2

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1. Introduction

PM2 is a lipid-containing bacteriophage that grows on BAL-31, a marine pseudomonas host [1]. This bacterial strain is able to utilize lactose, among several other sugars, as the only carbon source [2]. The existance of both a β-galactosidase activity and a β- galactoside transporting activity, tentatively identified as a lactose permease activity, has been described in BAL-31 cells [3]. The present work is a study of the β-galactoside transporting activity at different times after infection of the host cells with PM2. The results obtained show that prior to cell lysis there is a significant increase in transport activity, reflected in an increase in the maximum transport rates. The β-galactosidase activity remains unaffected prior to cell lysis to decrease slightly thereof. These results are discussed in terms of possible modifications of lipid membrane composition produced by PM2 infection.

2. Materials and methods

Isolated BAL-31 was grown in either lactose minimal medium or in EMS rich medium as described previously [3]. Cells were infected with PM2 at a m.o.i. of 15–20; PM2 bacteriophage was prepared as described elsewhere [1]. The ability of the intact cells or of the cell extracts to hydrolyze *O*-nitrophenyl β-D-galactoside (ONPG) was assayed as described [3]. To induce the cells grown in EMS medium, 5×10^{-4} M isopropyl β-D-thiogalactoside (IPTG) was added to the growth medium at the time of inoculation of BAL-31. The concentration of protein of the bacterial cultures was determined by the method of Lowry et al. [4], with bovine serum albumin as standard. ONPG and IPTG were obtained from Calbiochem.

3. Results and discussion

The effect of PM2 infection on bacterial cell growth in EMS medium plus 5×10^{-4} M IPTG is shown in fig.1A. Chloramphenicol was added to samples from the infected and the control cells at the times indicated in the abscissa. The turbidity of the cultures was measured at the time of chloramphenicol addition and after 20 min. This was done to avoid possible errors due to turbidity changes during the 20 min incubation period required to measure ONPG hydrolysis by the cell cultures. As shown in fig.1A, between 40 min and 50 min after phage infection, cell lysis starts to take place. It is worth noting that even in the presence of chloramphenicol there is a change in the turbidity of the infected cell culture during the 20 min incubation period (compare the solid squares and the solid triangles in fig.1A), indicating that the processes leading to cell lysis are not inhibited by chloramphenicol addition. The ONPG hydrolytic activity of intact cells, which reflects the transport of ONPG into the cells, and of the

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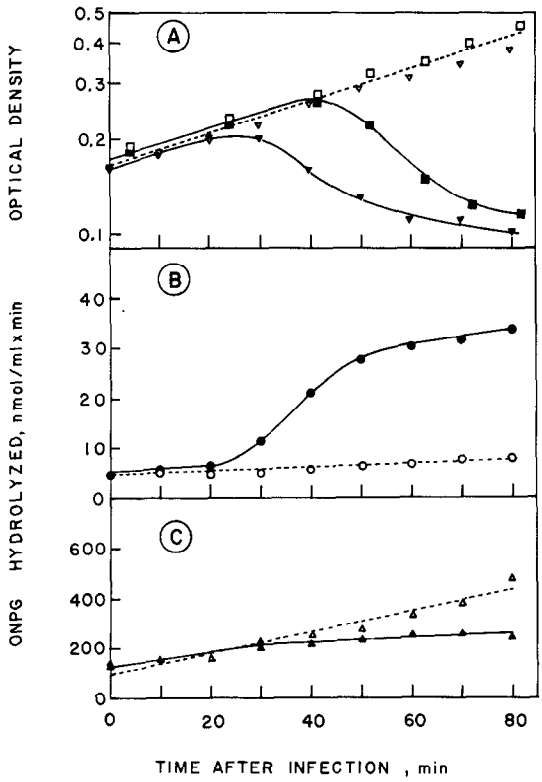

Fig.1A. Bal-31 cells were grown at 18°C on EMS medium with 5×10^{-4} M IPTG. At time zero the cells were infected with bacteriophage PM2 at a m.o.i. of 20. At the times indicated in the abscissa, chloramphenicol (400 μg/ml) was added to fractions of the cell culture, and the turbidity of the culture at the time of chloramphenicol addition (□,■) and 20 min afterward (▽,▼) was measured. (□,▽) Control; (■,▼) Infected cells.

Fig.1B. Fractions (0.5 ml) of the culture taken at the times indicated in the abscissa were assayed for the ability to hydrolyze ONPG by incubating with 6.7 mM ONPG for 20 min in the presence of 400 μg/ml chloramphenicol. (○) Control; (●) Infected cells. All other conditions the same as in fig.1A.

Fig.1C. Fractions (0.1 ml) of the culture to wich chloramphenicol was added (400 μg/ml) at the times indicated in the abscissa were shaken with chloroform for 30 s. The resulting solutions were assayed for the ability to hydrolyze ONPG by incubating with 6.7 mM ONPG for 20 min. (△) Control; (▲) Infected cells. All other conditions the same as in fig.1A.

cells infected with PM2, are shown in fig.1B as a function of time after infection. A net increase in the hydrolysis rate by the infected cells, as compared to the control cells, is apparent in samples taken at 30 min after infection, and reaches maximum levels in

Table 1
Effect of PM2 infection on the transport of ONPG by BAL-31 cells

Time after infection (min)	Culture turbidity[a] (A_{640})	Hydrolysis rate (nmol ONPG/ml × min)	
		Culture	Filtrate
20	0.270	5.9	0.5
23	0.300	6.1	0.5
26	0.305	6.5	0.5
29	0.300	8.6	0.5
32	0.295	13.0	0.5
35	0.290	21.8	19.0
38	0.280	24.4	24.5

[a]Measured 20 min after chloramphenicol addition

Cells were infected with PM2 at time zero at a m.o.i. of 15. At the times after infection indicated above, chloramphenicol (400 μg/ml) was added to 3.5 ml fractions of the culture. A fraction (0.5 ml) of each sample was used to measure ONPG hydrolysis by incubating with 6.7 mM ONPG for 20 min at 27°C. After measuring turbidity at the end of the 20 min incubation period, the remaining 3.0 ml of the cell culture were filtered through HA (0.45 μm) Millipore filters. The hydrolysis of ONPG by the filtrates was measured under the same conditions described above for the cell culture

samples taken at 40–50 min after infection. This increase in the ONPG hydrolytic activity of the infected cells is not accompanied by changes in the hydrolysis rates of the corresponding cell extracts, which remain indistinguishable from the control until 30 min after infection and decrease slightly thereafter (fig.1C).

Table 1 shows the results of a similar experiment in which cells grown in EMS medium with IPTG were infected with PM2 at time zero. Shorter time intervals were used in this case. At 29 min after infection there is already an increment in the hydrolysis of ONPG by the infected cells, without a significant change in the turbidity of the culture. As a more sensitive test for possible cell lysis, fractions of the infected cell culture were taken simultaneously with the samples to measure ONPG hydrolysis and, after incubation for 20 min in the presence of chloramphenicol, they were filtered through HA Millipore filters (0.45 μm) and the ability of the filtrates to hydrolyze ONPG was measured. Only 35 min after infection (table 1), significant amounts of β-galactosidase activity were released to the filtrate. These results suggest that the early increment (30 min after infection) in the ONPG hydrolytic activity of the infected cells grown in EMS with IPTG is due to a property of the transport system itself and is not caused by a release of the intracellular β-galactosidase to the reaction medium.

The results shown in fig.2 strenghten this point. Cells grown in EMS medium with 5×10^{-4} M IPTG were infected with PM2. Chloramphenicol was added at 32 min after infection to stop cell growth and the hydrolysis of ONPG by the resulting cell culture was measured as a function of ONPG concentration. It has been shown previously [3] that while BAL-31 cells grown in minimal lactose medium display both a β-galactosidase and a β-galactoside permease activity, in cells grown in EMS medium with IPTG only the β-galactosidase is present so that ONPG enters the cells by diffusion. In contrast to these findings, the infected cells grown in EMS with IPTG have a large transport component for ONPG, fig.2. Such a drastic change, from a system in which no transport activity was detectable to a system with a V_{max} of about 200 nmol ONPG/mg × min, indicates that as a result of phage infection a substantial change in the behavior of the transport system has taken place.

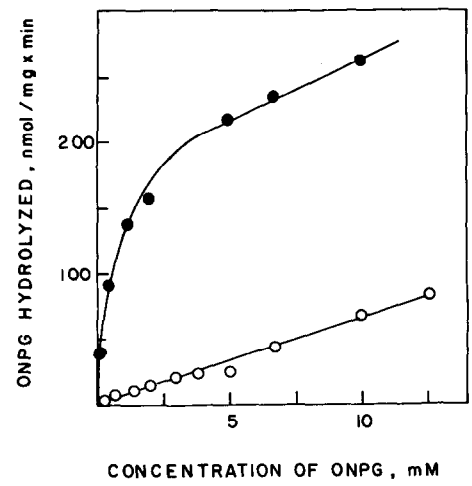

Fig.2. BAL-31 cells were grown at 18°C on EMS medium with 5×10^{-4} M IPTG. The exponentially growing culture was infected at time zero with PM2 at a m.o.i. of 20. At 32 min after infection, 400 μg/ml chloramphenicol was added to stop cell growth. Fractions (0.5 ml) of the resulting culture were assayed for the ability to hydrolyze ONPG as a function of substrate concentration. (○) Control; (●) Infected cells.

A similar type of experiment was carried out with cells grown in lactose minimal medium, which display both a transport component and a diffusion component for the entry of ONPG into the cells [3]. In this case the transport of ONPG has increased from a V_{max} of 330 nmol ONPG/mg × min in the control cells to 840 nmol/mg × min in the infected cells, after substracting the corresponding diffusion components, fig.3A. The double reciprocal plot of the results shown in fig.3A for the hydrolytic activity of ONPG of the infected cell culture after substracting the diffusion components, versus the reciprocal of the ONPG concentration is shown in fig.3B. Both control and infected cell cultures have the same apparent K_m for the transport of ONPG, 0.4 mM; they differ in that the maximum rate of transport is 2.5 times higher in the infected cells than in the control cells.

Two different experimental results can be considered at this point to rule out the possibility that the increase in ONPG transport is due to an artifact caused by cell membrane leakiness produced by infection, which would allow the intracellular β-galactosidase to react with ONPG giving high values for the hydrolytic activity.

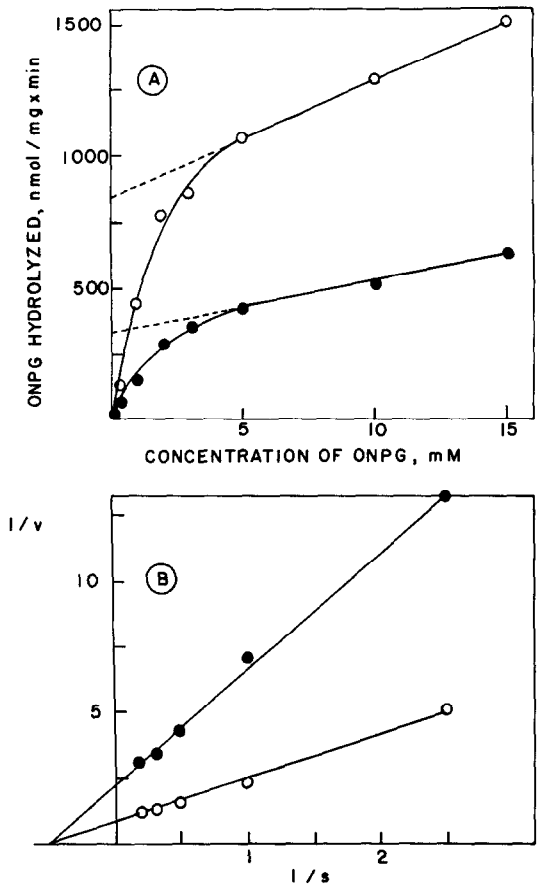

Fig.3A. BAL-31 cells were grown at 28°C in minimal lactose medium. The exponentially growing culture was infected at time zero with PM2 at a m.o.i. of 20. At 40 min after infection, 400 μg/ml chloramphenicol was added to stop cell growth. The resulting cultures were assayed for the ability to hydrolyze ONPG by incubating 0.5 ml fractions with variable ONPG concentrations for 20 min. (•) Control; (○) Infected cells.

Fig.3B. Double reciprocal plot of the ONPG hydrolytic activity of control and PM2 infected BAL-31 cells grown on lactose minimal medium. The data are taken from the experiment shown in fig.3A, after correcting for diffusion. (•) Control; (○) Infected cells.

1. Samples of the infected cell cultures treated with chloramphenicol were filtered through Millipore filters (HA, 0.45 μm) at the end of the experimental assays such as those shown in fig.2 and 3. In no case was any β-galactosidase activity detectable in the filtrates indicating that the infected cells still represent a permeability barrier for β-galactosidase.

2. As shown in fig.2 and 3A the infected cells retain a diffusion component for the entry of ONPG, which in the cells grown in EMS with IPTG is 1.4 times larger than in the control and in the cells grown in minimal lactose medium is 2.2 times larger than in the corresponding control.

In both cases the transport component is at least 20 times larger than the diffusion component in the infected cells, indicating that the infected cells still represent a permeability barrier for ONPG.

It is possible to assume then that the increase in the transport of ONPG in the infected cells is a reflection of a modification in the transport system itself. The finding of the same apparent K_m values for ONPG transport in control and infected cells suggests that the affinity for ONPG remains unchanged after infection and that the increase in transport rates might be due to either a modification in the rate-limiting step of the reaction or to an increase in the number of transporting units. The latter alternative seems unlikely. As shown in fig.1C, the β-galactosidase activity of the cells at 30 min after infection is not different from the activity of the uninfected cells, ruling out the possibility of an increase in the number of transporting enzyme units if both enzymes are coded by the same operon, as the available evidence seems to indicate [3]. A modification of the rate limiting step of the transport reaction, probably the translocation of ONPG across the membrane, seems the most likely explanation.

It is known that the lipid composition of phage PM2 is different from that of the host cell and that prior to lysis a substantial modification in the lipid composition of the host takes place [5]. It has been reported in other systems that large changes in the activity of membrane enzymes take place as a result of modifications in the membrane lipid composition [6,7]. The observed changes in the rates of ONPG transport after infection might be due to changes in the lipid composition of the BAL-31 membrane, although other explanations are possible. Recent experiments with *Escherichia coli* infected with phage T7 have shown that at early times after infection there is a phage-coded modification of the bacterial membrane that results in an increase of its fluidity

[8]. The elucidation of the mechanism by which phage PM2 produces an increase in the transport of ONPG of the host prior to lysis needs further experimental analysis.

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