VIRUS INFECTION AFFECTS THE MOLECULAR PROPERTIES AND ACTIVITY OF GLUCOSE-6-P DEHYDROGENASE IN *ANACYSTIS NIDULANS*, A CYANOBACTERIUM

Novel aspect of metabolic control in a phage-infected cell

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1. Introduction

Infection of a bacterial cell with bacteriophage is well known to result in a dramatically altered pattern of nucleic acid and protein synthesis (or breakdown) in the host cell. In contrast, it has been stressed that the respiration of the host is not affected by phage infection [1], except at the onset of lysis [2]. Study of enzyme levels in cell-free extracts [3], and investigation of the path of carbon in vivo [4] also revealed little effect of phage infection on the activities of respiratory enzymes. Thus it has been asssumed that the pre-existing respiratory machinery of the host cells can supply energy and C-skeletons in a suitable form and in sufficient amount for the new synthetic processes initiated by bacteriophage attack.

All the work leading to the above conclusions has been done on heterotrophic bacteria, mainly *Escherichia coli*, infected with DNA phages. No information on the effect of phage infection on the respiratory enzymes or metabolism of autotrophic prokaryotes is available.

The cyanobacteria are a major group of photosynthetic prokaryotes [5]. Some members of the group are attacked by specific bacteriophages (cyanophages) [6]. Therefore the cyanobacterium/cyanophage system seemed promising for the investigation of the effect of phage infection on the respiratory metabolism of an autotrophic cell.

We show here that the respiratory metabolism of *Anacystis nidulans*, a unicellular cyanobacterium, is drastically altered upon phage infection.

2. Materials and methods

Anacystis nidulans cells were grown and infected by cyanophage AS-1 [7] at a multiplicity of infection of 5, as described [8]. The samples, at the times indicated in the experimental section, were centrifuged, suspended in a 3-fold vol. of ice cold 50 mM Tris–HCl buffer (pH 7.5) and sonicated under cooling. If not indicated otherwise, 40 000 × *g* supernatants were used in the assays, the details of which are described in the legends to the figures.

3. Results

3.1. *Glucose-6-P dehydrogenase (G6PDH) activity increases upon phage infection*

The activity of G6PDH in *A. nidulans* cells infected by cyanophage AS-1 rapidly increases after infection (fig.1a). This is an 'early' response of the host since, under the experimental conditions used, phage adsorption requires 20 min for completion [9] and the lytic cycle lasts for 5–6 h [8,10]. *A. nidulans* cells mock-inoculated with heat-killed or ultraviolet-inactivated AS-1 phage and *Escherichia coli* B cultures inoculated with T4 phage were used as 'controls'. The inactivated phage had no effect on the G6PDH activity in *Anacystis*. In *E. coli* the G6PDH activity declined after infection with T4 phage (data not shown). Chloramphenicol (CAM), rifampicin, and dichlorophenylmethylurea (DCMU) had little or no effect on the increase in G6PDH activity in the

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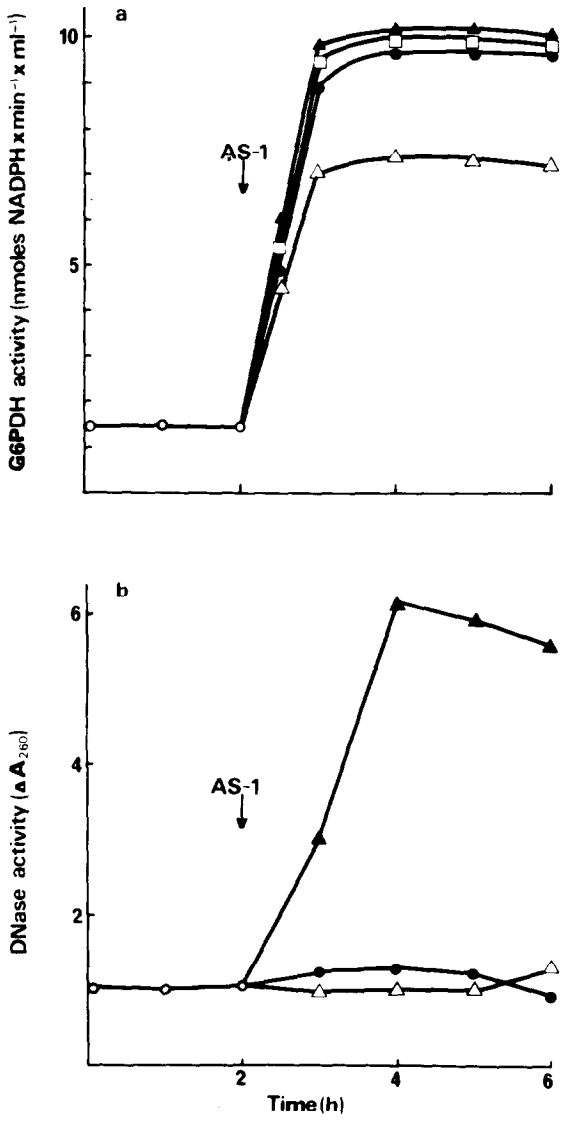

Fig.1. Enzyme changes in *Anacystis nidulans* infected by cyanophage AS-1. (a) Activity of G6PDH extracted from the cells, at intervals, after infection. V_i values (velocity of the reaction during the first min) obtained before infection (○) and after infection are shown for cells cultured in the absence of inhibitors (▲) and in those treated with 0.01 mM DCMU (□), 0.3 mM CAM (△), and 0.1 mM rifampicin (●), respectively. The inhibitors were added to the cultures simultaneously with the phage. The reaction system contained 2.5 mM G6P, 0.15 mM NADP, 5 mM $MgCl_2$, 50 mM Tris–HCl at pH 7.5, and 40 000 × *g* supernatant (10 μl) corresponding to 2 × 10^8 cells. The ΔA_{340} was recorded at 26°C. (b) DNase activity measured in aliquots of the same samples. A standard assay system was used as described [21].

infected cells (fig.1a). CAM and rifampicin inhibited the incorporation of labeled precursors into protein and RNA, respectively, up to 90%. 'Internal controls' were used to check whether the inhibitors affected the development of other virus-induced enzyme activities in our system. Figure 1b shows that CAM and rifampicin inhibited the phage-induced increase in DNase activity [8] by 90–100%. The same is true for the virus-induced lysozyme activity (not shown). Thus at least a major part of the phage-induced change in G6PDH activity appears to be due to enzyme activity rather than new enzyme protein synthesis.

3.2. *The properties of cyanobacterial G6PDH*

As to a possible explanation for the infection-induced change in G6PDH activity, a recent paper [11] on the G6PDH of *Anabaena* sp., a cyanobacterium, is relevant. It was shown that the G6PDH of *Anabaena* is a hysteretic enzyme. These enzymes undergo slow, reversible transitions between different aggregation (oligomeric) states which differ in catalytic activity [12]. The equilibrium between the cyanobacterial G6PDH forms is shifted in favour of the more active, oligomeric forms at high enzyme concentration, high levels of the substrate, G6P, and at slightly acidic pH. NADP (in certain concentrations) and alkaline pH shift the equilibrium in the opposite direction. As a result of an interplay of these factors the enzyme can be present in three different kinetic states. As defined [11], the oligomeric form is in a 'hyperactive' state characterized by an initial burst of enzyme activity whereas the monomeric form is in a 'hypoactive' state characterized by a lag; in both cases a constant and equal rate is established during the assay. The enzyme in the 'normal' state exhibits steady-state kinetics.

We have found that the properties of G6PDH of *Anacystis* and *Anabaena* are similar, and attempted to establish in which molecular and/or catalytic form the enzyme is present in the healthy and infected *Anacystis* cells.

3.3. *G6PDH forms in cyanophage-infected* Anacystis

To keep the in vitro concentrations of proteins and small molecular weight substances close to the in situ levels, we extracted the *Anacystis* cells with as small amounts of buffer as possible at pH 7.5,

because in the cytoplasm of light-grown *A. nidulans* the pH, as determined in vivo, has been reported to be 7.4–7.5 [11,13].

The enzyme obtained from healthy cells showed the 'hypoactive' type of kinetics whilst that extracted from the infected ones exhibited the 'hyperactive' type (fig.2a). When, before the enzyme assay, we preincubated extracts from healthy cells with G6P for 30 min or shifted the pH of the extract to 6.5 for the same period the enzyme exhibited the 'hyperactive' type of kinetics. Alternatively, dilution of the extract from infected cells, with no substrate added, and incubation of the diluted solution for 10 min before assay, converted the enzyme into the 'hypoactive' form (fig.2b). These results suggested that the G6PDH might be present in the infected cells mainly in the hyperactive form and in the healthy cells mainly in the hypoactive form. To test this idea, we analysed the G6PDH present in the extracts by sucrose density gradient centrifugation. This technique enabled the separation [11] of the different enzyme forms and the demonstration that the conversion of one form to another, by shifting the pH or adding G6P to the enzyme-containing solution, results in the expected change in the apparent molecular weight of the cyanobacterial G6PDH, corresponding to the postulated monomer ⇄ oligomer transitions. As shown in fig.3, the enzyme extracted from infected cells sedimented faster in the sucrose gradient than the enzyme from the healthy cells. After a shift of the pH of the extracts from healthy cells to pH 6.5, followed by incubation at this pH for 30 min, an enzyme form was obtained which had the same sedimentation characteristics as the G6PDH extracted from infected cells. Preincubation of the extracts from healthy cells with G6P before centrifugation had the same effect.

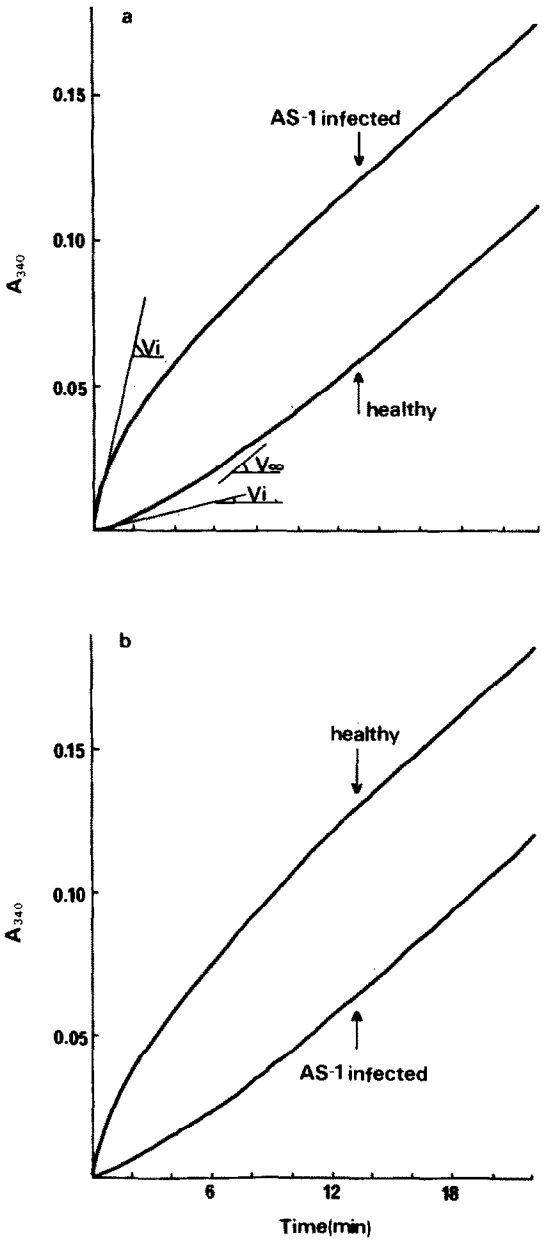

Fig.2. Effect of phage infection on the kinetics of G6PDH from *A. nidulans*. (a) Direct recording of the enzyme activity in extracts obtained from cells 3 h after infection and from corresponding controls. The assay system contained 10.0 mM G6P, 0.5 mM NADP, 10 mM $MgCl_2$, 10 mM β-mercaptoethanol, and 50 mM Tris–maleate buffer at pH 7.5. 40 000 × *g* supernatant corresponding to 2×10^8 cells (10 μl) was added to the reaction mixture to start the reaction (1 ml final vol.). (b) An aliquot of the extract from healthy cells was preincubated for 30 min with 10 mM G6P, 10 mM $MgCl_2$ and 10 mM β-mercaptoethanol in 50 mM Tris–maleate buffer (pH 7.5). After preincubation, 10 μl of this solution was assayed for G6PDH activity in 1 ml of the complete reaction system described for fig.2a. The reaction was started with the addition of 0.5 mM NADP. The extract from the infected cells (10 μl) was diluted 80-fold in 50 mM Tris–maleate buffer (pH 7.5) and preincubated for 10 min before assay in 1 ml final vol. of the complete reaction system.

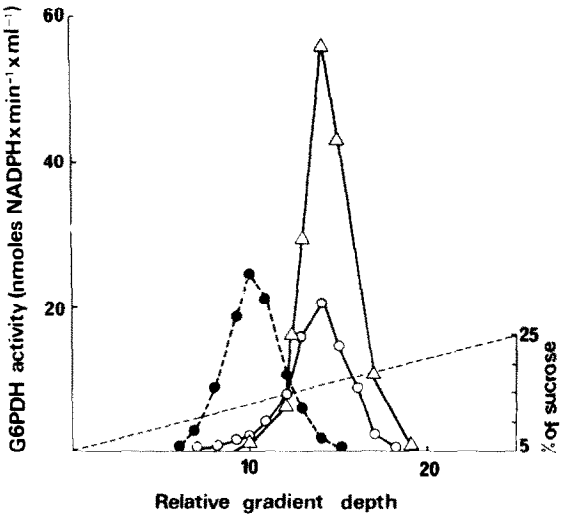

Fig.3. Sedimentation profile in sucrose density gradient of G6PDH from healthy and phage-infected *Anacystis* cells. Aliquots (0.9 ml) of the 10 000 × *g* supernatants of crude extracts (pH 7.5) were layered on linear sucrose gradients (5–25%, w/v) and spun at 4°C for 9 h in the SW 50.1 rotor in a Beckman L2-65B ultracentrifuge at 33 000 rev./min. Fractions (0.2 ml) were collected and assayed for G6PDH activity, using the reaction system described in the legend to fig.1. Extract from healthy (•) and infected (○) cells. Extract from healthy cells, preincubated at pH 6.5 for 30 min, and layered on a sucrose gradient also adjusted to pH 6.5 (△).

The fractions from the sucrose gradients, corresponding to the peaks of G6PDH activity, were pooled and assayed for the kinetic behaviour of the enzyme forms separated. The faster sedimenting enzyme exhibited the 'hyperactive' type of kinetics. The slower sedimenting enzyme was found to be in the 'hypoactive' form.

These results strongly suggest that the virus infection leads to a shift in the equilibrium between the hypoactive ⇄ normal ⇄ hyperactive states of G6PDH.

3.4. *Operation of the oxidative pentose phosphate pathway in the infected cells*

We have studied the breakdown of glucose in healthy and phage-infected *Anacystis* cells by using isotope techniques. Although the determination of the so called 'C_6/C_1-ratio' cannot yield absolutely quantitative results, the preferential liberation of $^{14}CO_2$ from the C_1 carbon of glucose, as compared to the release of $^{14}CO_2$ from the C_6 carbon, is usually taken as an indication of glucose breakdown predominantly via the oxidative pentose phosphate pathway (for cyanobacteria see [14–16]). We have found a steady increase in the rate of liberation of the C_1 as compared to the C_6 carbon atom of glucose fed at intervals to *Anacystis* cells during the infection process (fig.4). These results suggest the operation of a stimulated oxidative pentose phosphate pathway in the infected cyanobacterial cells. In *E. coli* infected with T phages, an unaltered carbon pathway [4] and a possible decrease in pentose phosphate shunt activity [17] have been reported. We have also found no increase in the rate of C_1-decarboxylation in *E. coli* B infected by T4 phage (data not shown).

The level of G6PDH activity is not the only factor in the regulation of the oxidative pentose phosphate pathway. The rate of reoxidation of NADPH produced by the NADP-specific dehydrogenases of the pathway may be equally or even more important. We have

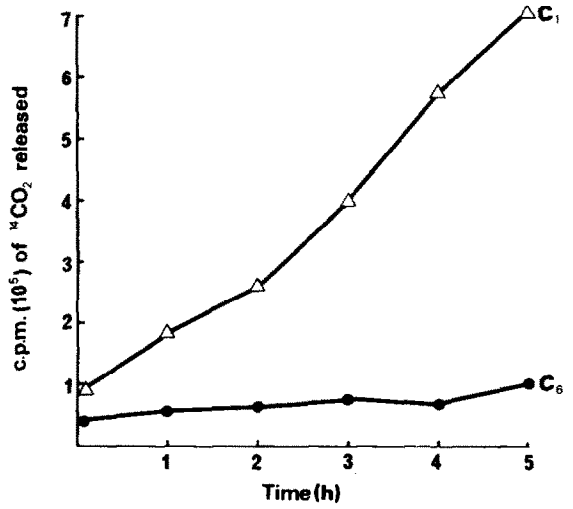

Fig.4. The release of carbon as $^{14}CO_2$ from the C_1 and C_6 C-atoms of glucose after infection of *Anacystis nidulans* with cyanophage AS-1. *A. nidulans* was cultured as described [8] and infected with AS-1 phage at a multiplicity of infection of 5. Aliquots (3 ml) of the culture were pulse labelled at intervals, with D-[1-^{13}C]glucose, and D-[6-^{14}C]glucose, respectively, during the infection process (0.5 μCi sample; 58 and 53 mCi/mmol, respectively). The CO_2 released was absorbed by 1 ml hyamine hydroxide in the centre well of the reaction vessels. The reaction was stopped after 50 min by adding 1 ml 2 N HCl. The radioactivity was measured in a liquid scintillation spectrometer.

found that the rate of enzymatic oxidation of NADPH was 5–6-times higher in 10 000 × *g* (membrane-containing) supernatants from infected *Anacystis* cells than in those from healthy ones (data not shown). Thus, enough NADPH may be reoxidized in the infected cells to keep the NADP-dependent dehydrogenases of the pentose phosphate shunt active.

4. Discussion

These results strongly suggest that phage infection alters the respiratory metabolism of the cyanobacterium studied. A major aspect of the alteration seems to be an activation of the oxidative pentose phosphate pathway. A number of factors are apparently responsible for this change. The activity of G6PDH, the first enzyme of the pathway, is greatly increased in the infected cells, due to a transition of the enzyme from 'hypoactive' to 'hyperactive' form. The exact mechanism of this transition remains to be elucidated. One effector may be G6P that accumulates in the phage-infected cell (data not shown) and may shift the equilibrium of G6PDH forms in favour of the hyperactive oligomer(s). In addition, the inhibition of G6PDH activity in the light, a characteristic feature of cyanobacterial metabolism [14,18,19], appears to be lifted by cyanophage attack. This inhibition is due mainly to the photosynthetic production of NADPH (and perhaps ATP), potent inhibitor(s) of the cyanobacterial G6PDH [18–20]. The NADPH produced in the virus-infected cells may be reoxidized by a highly active NADPH-oxidase system. In addition, other NADPH-consuming, virus-specific processes, such as the increased formation of deoxyribonucleotides from ribonucleotides, may contribute to a higher rate of reoxidation of NADPH.

The changes observed appear to be 'advantageous' for the phage. In *A. nidulans* infected with the AS-1 phage the total amount of DNA increases to a level which is 4-times higher than the DNA content of the uninfected cells (data not shown). The breakdown products of host DNA may not be sufficient to meet the requirements for building blocks (pentose skeletons, nucleotides) of phage DNA synthesis. Most likely, the photosynthetic products (via G6P) are channeled into the oxidative pentose phosphate pathway.

The results described are in contrast to all the relevant observations on heterotroph host/bacteriophage combinations. We suggest that the difference is closely connected with the intrinsic differences in the control of carbon metabolism in the two groups of organisms and that the phage-induced alteration of respiratory metabolism in cyanobacteria is specifically associated with the properties of the cyanobacterial G6PDH.

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