Differential Effects of Visible Light on Active Transport in *E. coli**

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ABSTRACT

Light of wavelengths above 400 nm inactivated several active transport systems in *E. coli* ML 308. Rates of inactivation for uptake of threonine, glycine, leucine and methionine were similar and differed from those for methyl thio-β-D-galactoside and phenylalanine. These differential effects indicate that inactivation of the threonine, glycine, leucine and methionine systems is linked to a common photochemical lesion differing from that involved in the inactivation of the methyl thio-β-D-galactoside and phenylalanine systems. These lesions may serve as labels to identify molecules involved in transport or energy coupling processes.

INTRODUCTION

There is continuing interest in the development of methods to identify components of bacterial membrane transport systems and their associated energy coupling mechanisms(1). The present work suggests that a useful approach is through the study of photoeffects on transport.

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Abbreviations: gly = glycine, leu = leucine, ONPG = o-nitrophenyl β-D-galactoside, phe = phenylalanine, TMG = methyl thio β-D-galactoside, thr = threonine.

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MATERIALS AND METHODS

Culture Conditions. *E. coli* ML 308 (i^- y^+ z^+ a^+) grown at 37°C on medium 63(2) supplemented with 0.4% glycerol was maintained on glycerol-medium 63 agar. Cells from an overnight liquid culture were inoculated into fresh medium to an optical density (600 nm) of 0.3-0.4 (Beckman DB spectrophotometer, 1 cm path length) and grown to an optical density of 0.7-0.8 (mid log) in a gyrotory shaker. Cells were harvested by centrifugation (3000xg, 5 minutes) washed once and suspended in growth medium to 1/4 the original volume (0.8-1.0 mg/ml dry weight). Cells were then used immediately for illumination.

Preparation of Vesicles. Vesicles were prepared as described by Kaback(3) and stored in liquid nitrogen (protein concentration = 3 mg/ml).

Illumination. Cell or vesicle suspensions were illuminated under aerobic conditions using a 450 watt mercury arc filtered to exclude light below 400 nm. Incident light intensity was 200 mw/cm^2. For anaerobic conditions, O_2-free nitrogen was bubbled through the suspension. Chloramphenicol (80 ug/ml) was added to the cells immediately after illumination.

Transport Assays. Initial rates of uptake were taken to be equal to net uptake 30 seconds after addition of radioactive substrate. A 1/2 ml aliquot of illuminated cells was added to 1.5 mls of medium-63 plus glycerol and radioactive substrate and incubated with shaking for 30 seconds. A 1.0 ml sample was then rapidly filtered using a 0.45 μ millipore filter and washed with 5 mls of medium 63 at room temperature. Assays using vesicles were carried out as described by Kaback(3). Radioactivity on the dried filters was measured in a scintillation counter using Aquasol as scintillation fluid. The concentration

of [^{14}C]-TMG, 10^{3} M, was equal to 1.7 Km, determined as 6×10^{-4} M. Concentrations of ^{14}C amino acids used ranged from 5 to 50 times Km(4-6).

Viability. Viability was measured by the plate count technique using nutrient agar; colonies were counted after 18 hours incubation at 37°C.

Enzyme Assays. Activities of β-galactosidase, glucose 6-phosphate dehydrogenase, and l-malate dehydrogenase were assayed according to the methods of Kepes(7), Scott and Cohen(8) and Cartwright and Henning(9) respectively, using cells sonicated for 4 min at 0°C with a Branson Sonifier cell disruptor.

RESULTS

After 10 minutes of illumination uptake of threonine decreased to ~5% that in unilluminated controls (Figure 1A). Phenylalanine uptake was unaffected for about 3 minutes after which inactivation proceeded rapidly but at a rate only about one-half that for threonine. Uptake of TMG began to decrease immediately at a rate comparable to that for phenylalanine after three minutes of illumination. Leucine, methionine and glycine uptake, all of which probably occurs largely by transport systems differing from that for threonine, phenylalanine and TMG(3-5) exhibited inactivation kinetics virtually identical to that for threonine (Figure 1B).

The effect of light is relatively specific for transport processes. Although 10 minutes of illumination resulted in a marked decrease in rates of uptake (Figure 1) it had little effect on viability (Figure 2) and enzymes such as l-malate dehydrogenase and glucose-6-phosphate dehydrogenase were inhibited by 15% or less. The activity of β-galactosidase decreased by < 5% after 20 minutes of illumination.

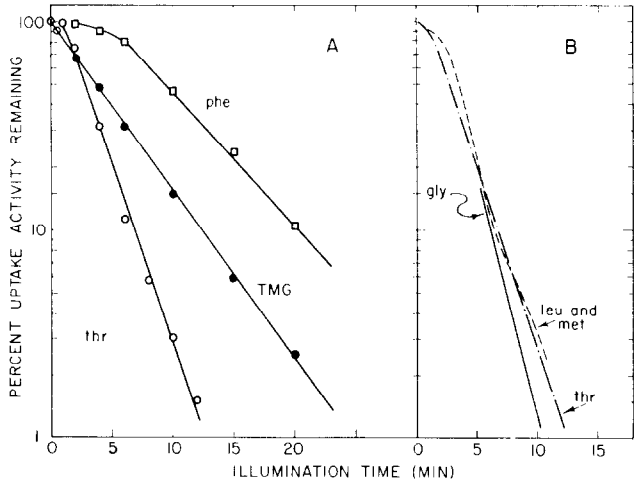

Figure 1 A: Typical photoinactivation of uptake of phenylalanine, TMG and threonine by *E. coli* ML 308. Uptake level of 100% is that of unilluminated controls.

B: Photoinactivation of uptake of threonine, leucine, methionine and glycine. The threonine curve is the same as that in part A.

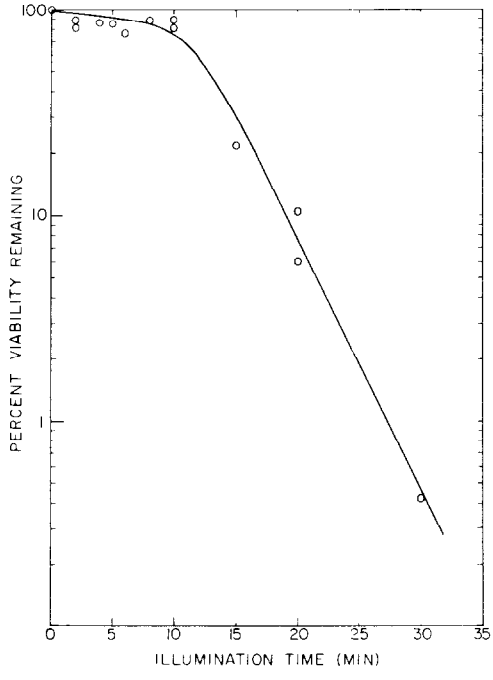

Figure 2 Photoeffects on cell viability.

Light inactivated ONPG hydrolysis, a result in accord with the TMG results. However, since β-galactosidase activity is not significantly altered, the inactivation indicates that the photochemical lesion prevents the lac permease from turning over rather than preventing accumulation of β-galactosides.

The decreases in uptake are not due to photochemically induced cell leakage. An increase in cell permeability would result in an increase in the rate of ONPG hydrolysis in the presence of dithiogalactoside, an inhibitor of the lac permease (7). No evidence for such an increase was found for illumination times up to 20 minutes.

The photoeffects depend markedly on the presence of oxygen. In its absence, the rate of TMG uptake remained unchanged while that for glycine and phenylalanine decreased by < 15% after 15 minutes illumination and no significant decrease in viable counts could be measured. The oxygen requirement indicates that inactivation is a photodynamic process (10). In such processes the initial event is absorption of light by a photosensitizer which is then deactivated by a series of reactions involving molecular O_2 at some point. The photosensitizer affecting TMG uptake in cells is probably membrane bound since similar photo-inhibition effects were found in vesicles. Illumination effects on amino acid uptake in vesicles were not investigated.

DISCUSSION

The differential photoinactivation of transport systems may provide a useful method for obtaining information about molecular aspects of transport since the underlying photochemical lesions could serve as labels or markers to identify molecules

involved in transport. A salient point in this regard is the finding that several amino acid transport systems (threonine, glycine, methionine, leucine) which probably utilize different carriers show identical inactivation kinetics which differ from those for TMG and phenylalanine. Thus, the former amino acid systems possess at least one step employing a common molecular feature different from that for the TMG and phenylalanine systems which is sensitive to photodynamic action. The nature of this common feature is of obvious interest. One possibility is that the photochemical damage occurs to a common characteristic of the carrier molecules or to a closely associated moiety. Another is that damage occurs at some step in an energy coupling process common to the amino acid systems but not to that for TMG or phenylalanine. The report that different energy coupling systems may be employed by different transport systems in *E. coli* (11) is of interest in this regard.

Although the site of the lesion for the systems exhibiting similar kinetics of inactivation is not known, the site for inactivation of β-galactoside uptake probably involves a carrier molecule. Previous studies (12) have shown that inhibition of energy yielding reactions inhibits accumulation of β-galactosides without drastically affecting turnover of the permease. Hence, the observation that illumination decreases ONPG hydrolysis without significantly affecting β-galactosidase activity points to photochemical damage at the carrier level.

The endogenous photosensitizer(s) are not known. Quinones are candidates since they can absorb in the wavelength region employed and can be modified by light to inactivate transport in cell fractions (13,14,15); flavins and cytochromes may also be involved. Work is currently in progress to identify

the photosensitizer(s) and the photochemically induced lesions.

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