JOURNAL OF BACTERIOLOGY, Apr. 1976, p. 140-146
Vol. 126, No. 1

Printed in U.S.A.

Inactivation of Membrane Transport in *Escherichia coli* by Near-Ultraviolet Light

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Received for publication 19 November 1975

Evidence is presented that near-ultraviolet (near-UV) light can alter galactoside transport in *Escherichia coli* in several independent ways. It can inactivate the permease system per se, it can interfere with metabolic energy production or transfer, and it can cause an increase in the generalized permeability of the membrane. Earlier publications suggested that near-UV destroys cofactors needed for electron transport and thus places a limitation on energy reserves. In agreement, we found that the active accumulation of [^{14}C]thiomethyl-β-D-galactopyranoside is decreased after irradiation by a larger factor than that due to action directly on the permease system. The effect on the latter was measured by the decrease in the rate of *o*-nitrophenyl-β-D-galactopyranoside (ONPG) transport. As evidence that energy supplies for this "downhill" process did not become rate limiting after irradiation, we found that carbonylcyanide-*m*-chlorophenylhydrazone did not stimulate ONPG transport of irradiated cells. Cells genetically deficient in functional permease or cells treated with formaldehyde still transport ONPG passively, although at much lower rates. With the use of such cells, it was found that high fluences (doses) made the cells leaky. Further evidence that the permease system and the metabolic energy system can be inactivated independently is also presented. It is shown that a photoproduct from the irradiation of chloramphenicol inactivates the permease system much more efficiently than the energy system. In addition, it is shown that thio-β-D-digalactopyranoside protects the permease system, but not the energy system, both against direct inactivation by near-UV and against photosensitized inactivation in the presence of chloramphenicol.

Recently there has been a renewed interest in the effects of visible and near-ultraviolet (near-UV) light on biological systems. It has been found that broad-spectrum near-UV can block the electron transport chain by photochemical decomposition of aromatic cofactors, predominately the membrane-bound quinones (8, 9, 19; see review by Jagger [7]). Near-UV also inactivates the transport capability of *Escherichia coli* membrane vesicles for succinate and proline (14) and impairs accumulation of glutamate in *Bacillus licheniformis* membrane vesicles (15). It has also been shown that the transport capability of intact *E. coli* can be decreased by intense visible light (4). We report here that near-UV inactivates the functioning of the galactoside permease system of *E. coli* in several distinct and independent ways.

The galactoside permease system offers many advantages over other transport systems for the localization of lesions because it is possible to distinguish effects on the permease system itself from effects on the production and coupling of metabolic energy to the permease system (12, 13). For example, a treatment interfering with energy metabolism would block the "uphill" accumulation of radioactive thiomethyl-β-D-galactoside (TMG) against a concentration gradient much more than it would block the "downhill" in vivo cellular hydrolysis of *o*-nitrophenyl-β-D-galactopyranoside (ONPG) where the flux is with the concentration gradient. The destruction of the transport system itself lowers both assays. It can be assessed with the in vivo ONPG assay, independently of changes in the energy status of the cell. The in vivo ONPG assay system has minimal dependence on the energy status of the cell (12) and can be made fully independent of the cell's energy metabolism (4) by the addition of carbonylcyanide-*m*-chlorophenylhydrazone (CCCP). This proton conductor converts both normal cells and energy-depleted cells to the same

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quasi-facilitated diffusion system: an ONPG molecule and a proton concomitantly bind to the carrier (symporter) (20) and traverse the membrane, independently of previous energy supplies. We designate this CCCP-treated system "quasi-facilitated" because two membrane carriers, the symporter and CCCP, are needed for continuing transport, but coupling to the cell's energy metabolism is prevented (4). With near-UV, we find that the permease and its energy supply are inactivated independently. Independent evidence for separate action on permease and energy supply is also presented, obtained from the studies of the effects of a protective agent (thio-β-D-digalactopyranoside, TDG), a sensitizer (chloramphemicol, CAP), a permease inactivator (formaldehyde), and a mutant defective in permease.

MATERIALS AND METHODS

The organisms used were *E. coli* B/r (originally obtained from E. Witkin) and *E. coli* ML35. The former has a wild-type operon and must be induced to have permease and β-galactosidase; the latter is constitutive and produces β-galactosidase in the absence of inducers but does not produce functional permease. Cultures were grown aerobically at 35 to 38 C with 0.2% glycerol in M9 buffer (17) under dim laboratory illumination. The cultures were induced with 5×10^{-4} M isopropyl-thio-β-D-galactopyranoside (IPTG) for three to five generations prior to harvest. For irradiation or measurements of uptake, exponentially growing cells were harvested by centrifugation at dry cell weights of 0.07 to 0.1 mg/ml (absorbance at 600 nm, 0.2 to 0.3), washed twice, and suspended to an absorbance of 0.4 in M9 buffer at room temperature. For some of the energy depletion studies, however, induction was carried out with 5×10^{-3} M IPTG, and 0.2% D-glucose was added during the final generation.

[U-^{14}C]TMG, with a specific activity of 23.3 mCi/mmol, was obtained from Schwarz/Mann, Orangeburg, N.Y. IPTG and TDG were obtained from Sigma Chemical Co., St. Louis, Mo. CCCP was purchased from Calbiochem, La Jolla, Calif., and stored in 95% ethanol at −20 C.

The irradiations were carried out in a 1-cm water-jacketed Pyrex cuvette, with exposure to the light from four North American Phillips lamps, type HPW125 (125 W). The cuvette was positioned 1.5 cm from the face of each lamp. The relative emission spectrum of the lamps was determined previously by Peak and Peak (16) and is composed of three emission bands at 365, 334, and 313 nm. The 365-nm band accounted for more than 98% of the total blacklight output. The fluence rate was 30,000 J/m^2 per min. Dosimetry was performed with a calibrated radiometer (model 65, Yellow Springs Instrument Co., Kettering, Ohio). During irradiation, the cells were aerated with moist air and the temperature was maintained at 22 ± 2 C.

The [^{14}C]TMG transport assays were similar to those described by Robbins and Oxender (18). Suspensions were aerated for 10 to 15 min (in some cases in the presence of chloramphenicol at a concentration of 100 μg/ml) before irradiation and then exposed to radioactive TMG or amino acids prepared in the same M9 buffer.

Samples (50 μl) were membrane filtered, washed with 2 ml of buffer, dried, and counted in 5.0 ml of scintillation fluid containing 42 ml of Spectrafluor PPO-POPOP [2,5-diphenyloxazole−1,4-bis-(2,5-phenyloxazolyl)-benzene; Amersham/Searle, Arlington Heights, Ill.] and 100 g of naphthalene in 1 liter of dioxane. The uptake data are given as plateau values determined at 6 min; by this time the accumulation was maximum and time independent. Maximal values of uptake were always less than 5% of the available labeled substrate. Measurement of the time course of uptake of control and heavily irradiated cells showed that the time to reach half of the plateau value was constant, at approximately 1 min. Controls for binding of nonspecific radioactivity to cells and membrane filters were made by parallel measurements with cells pretreated with 30 mM azide.

The in vivo hydrolysis of ONPG was carried out as described previously (2) at 28.0 C with 1.85 mM ONPG, pH 7.0, in a Gilford recording spectrophotometer at 420 nm. Our previous work (1, 11-13) showed that the rate of hydrolysis of ONPG is a valid measure of cellular permease when β-galactosidase is present internally in excess and correction is made for non-permease-mediated hydrolysis. Controls for non-permease-mediated hydrolysis were run by the addition of neutralized HCHO to 10 mM.

The energy deletion procedure was carried out as described earlier (13). Briefly, the growing cells were washed free from exogenous carbon sources, incubated with 20 mM α-methyl-D-glucopyranoside (α-MG) and 40 mM NaN_3, washed again, and suspended in buffer.

In experiments in which thiodigalactoside was present during the irradiation, the cells were washed once before the assay for controls or if 10^{-4} M TDG was used, and twice if a higher concentration was used. This routine was chosen because TDG is known to bind to the permease system with a K_m of 2×10^{-5} M (3, 10); this washing procedure was found to be adequate to eliminate the competitive inhibition of TDG on either assay. It also removed any photoproducts present in the medium.

RESULTS

Test of ability to dissociate energy metabolism from galactoside transport in strain B/r. Although it had been demonstrated that cells of *E. coli* ML and K-12 can be depleted of all energy reserves usable by the lactose transport system by treatment with α-MG and NaN_3 (4, 13) and that such cells can carry out downhill transport of ONPG when a proton conductor such as CCCP is added (4), it was necessary to extend those observations to strain B/r. It is more difficult to show the latter with noncon-

stitutive strains. The energy depletion procedure depends on: (i) the removal of exogenous energy sources; (ii) forcing the cells to carry out the gratuitous and energy-consuming group translocation of α-MG to form its 6-phosphate (the transport continues because there is dephosphorylation to and efflux of internal α-MG); (iii) stimulation of this process (at least initially) by azide; (iv) prevention of aerobic metabolism, which probably forces the cells to consume their energy reserves fermentatively. Since in other cases such fermentation products are known to appear outside the cells, they should be removed by the washing procedure.

Table 1 shows the results of two experiments in which the net rate of in vivo permease function (i.e., the observed rate of ONPG hydrolysis minus the rate with formaldehyde-treated cells) decreased after the energy depletion treatment. The procedure works only marginally with strain B/r grown with glycerol and 5×10^{-4} M IPTG. The hydrolysis rate did not fall to zero as with the constitutive ML strain grown in glucose as the sole carbon source as described previously (4). Nevertheless, residual activities were significantly increased by the addition of 40 μM CCCP, showing that cells of strain B/r can be depleted of energy and then can be stimulated for downhill transport by proton conductors. The second experiment established that 8 to 16 μM CCCP gave optimum twofold stimulation of B/r cells grown for the last generation in a solution of 0.2% glycerol, 0.2% glucose, and 5×10^{-3} M IPTG and then energy depleted. Further controls also showed that CCCP abolished uphill TMG transport in strain B/r as rapidly and completely as it does in other strains (4).

These results justify the use of in vivo hydrolysis in the presence of CCCP as a crude probe to determine if another agent (e.g., near-UV) has inactivated the permease whether or not it also inactivates the energy supply or its coupling to transport.

TABLE 1. *Stimulation of the rate of ONPG hydrolysis of energy-depleted cells by CCCP*

Depletion time (min)	Control	Net rate of in vivo ONPG hydrolysis[a] (μmol/g per min)		
		Depleted	Depleted cells + 40 μM CCCP	Depleted cells + 8 μM CCCP
50	108	29	47	
60[b]	24	7	10	19

[a] ONPG rates were corrected for nonspecific transport with a formaldehyde control.

[b] Cells induced in 5×10^{-3} M IPTG in glucose-glycerol medium for the last doubling. All assays reported were conducted in 40 mM NaN_3.

Near-UV inactivation of the permease system and of the energy supply for galactoside transport. Rates of inactivation of uphill transport (TMG assay) and downhill transport (ONPG hydrolysis assay) are shown in Fig. 1. The open squares indicate the observed plateau values (at 6 min) for accumulation of [^{14}C]TMG. The dashed line is the unweighted least-squares regression for these data on the logarithmic plot and shows that the ability to accumulate TMG was inactivated with a half-life of 8.6 ± 0.6 min. An earlier independent experiment gave 10.0 ± 2.5 min. The corresponding value for 1/*e* survival for the data in Fig. 1 on the fluence scale is 3.9×10^5 J/m^2. The similar regression (solid line) through the ONPG assay rates (filled squares) corresponds to a half-life of

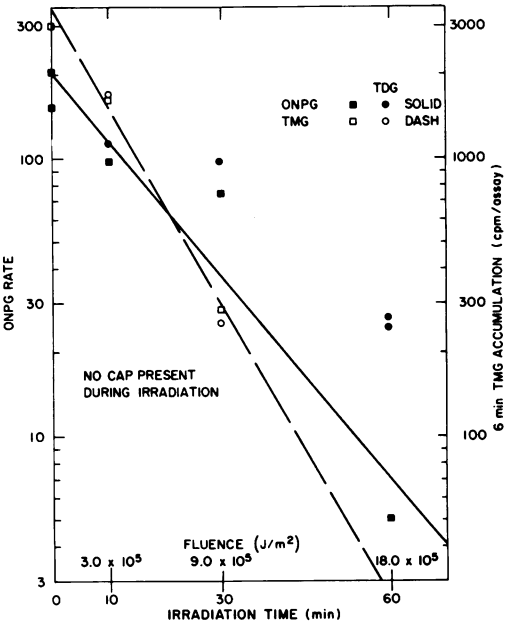

FIG. 1. *Inactivation of galactoside transport by near-UV. Values of transport activity include subtraction of the appropriate HCHO or azide control: downhill ONPG assay, solid symbols and left ordinate scale; uphill TMG assay, open symbols and right ordinate scale. The solid line is the least-square regression through the data points shown as closed squares, showing the effect of near-UV on the downhill assay. The dashed line is the regression through the values shown as open squares for the uphill assay. This line also passes through a pair of points at 60 min (not shown because the actual net counting rate for these samples was only 8 counts/min). TDG was used at 10^{-4} M for the data points indicated by circles.*

12.5 ± 2.1 min and an 1/e survival of 5.6×10^5 J/m^2. The results of two other independent experiments of this type gave ONPG transport half-lives of 19.4 ± 4.0 and 19.1 ± 5.8 min. The unweighted averages of these values show that the downhill transport process is considerably more resistant to near-UV (17.0 ± 2.2 min) than the energy-requiring process for uphill transport (9.2 ± 0.8 min).

As an integral part of the same experiment shown in Fig. 1, parallel rate measurements of ONPG hydrolysis were made in the presence of CCCP. The concentration of CCCP employed (10 μM) lowered the rate of ONPG hydrolysis of the unirradiated bacteria to 49% of the control, typical of previous experiments (4; A. L. Koch, unpublished data). Presumably this decrease occurs because CCCP eliminates the protonmotive force across the cytoplasmic membrane. All of the irradiated samples were also inhibited by CCCP to the same degree when compared with their corresponding controls. The average value after inhibition was 56 ± 7% (five data pairs) and shows that the downhill transport never became energy limited as the result of the irradiation. If energy depletion had limited the downhill assay, then CCCP should have stimulated transport instead of inhibiting it.

Protection by thiodigalactoside. Figure 1 also shows the effects of the presence of TDG at saturating concentrations during the irradiation. TDG had no appreciable protective effect on the inactivation of the ability of the cells to accumulate TMG (compare the two types of open symbols), but this digalactoside provided protection of ONPG hydrolysis capability (compare closed symbols) by about a fivefold factor after 60 min of irradiation.

Table 2 shows the results of another experiment in which the concentration dependence of TDG protection was explored more thoroughly. It can be seen that the protection was more evident at high fluences and the half-protective concentration was less than 10^{-4} M. The protection by TDG is specific in that another disaccharide, sucrose, did not protect. In addition, TDG did not protect leucine transport systems (data not shown). Since TDG did not protect the ability of the cell to carry out the uphill assay but did protect the downhill assay and since TDG is known to bind the permease, it can be concluded that the action of near-UV in destroying the ability to carry out the uphill assay is an indirect one and not inactivation of the permease system itself.

Effect of near-UV on passive permeability of the cytoplasmic membrane. The in vivo cellular hydrolysis assay can be used also as a technique to assess changes in membrane passive permeability (2, 11). Such changes can be detected with the use of mutant permeaseless strains carrying internal β-galactosidase, with cells in which the permease has been inactivated with HCHO, or with cells in the presence of specific inhibitors of the permease such as TDG. In all of these cases the rate of hydrolysis by cell suspensions was 10- to 20-fold less than with full levels of functional permease.

TABLE 2. *Thiodigalactoside protection against near-UV irradiation*

Irradiation time[a] (min)	TDG[b] (M)	Net rate of in vivo ONPG hydrolysis (μmol/g per min)
0	0	135
21	0	74
	10^{-4}	91
	10^{-3}	93
	10^{-2}	94
28	0	30
	10^{-4}	63
	10^{-3}	73
	Sucrose (10^{-3})	27

[a] Fluence rate, 3×10^4 J/m^2 per min.
[b] TDG removed before assay.

If near-UV causes cross-linking of membrane constituents, then the rate of cellular hydrolysis in any such system without functional permease should decrease. Alternatively, if near-UV alters the membrane structure to make the membrane more fluid or induces cracks or pores in the cytoplasmic membrane, then the rate should increase. Effects upon passive permeability to ONPG are indicated in Table 3 for both ML35 (a two-step mutant of ML30 which is both cryptic and constitutive) and for HCHO-treated cells of strain B/r. (Note, measurement of formaldehyde-treated cells was a standard control of every downhill permease in vivo ONPG assay, and these control values were subtracted to give net values.) Increased permeability was negligible for the first 30 min, although the cells did become leaky after illumination for 1 h. Thus, any increase in permeability that may have occurred could have had no significant effect on the observed inactivation of TMG accumulation by near-UV.

Effect of chloramphenicol during irradiation. While studying the specificity of TDG protection, we observed an anomalous increase in sensitivity to near-UV when the cells were irradiated in the presence of CAP. We observed that cells irradiated in the presence of CAP lost their transport activities about four times more

TABLE 3. *Effect of near-UV on membrane passive permeability*

Determination	Irradiation time (min)	Nonspecific permeation of membrane (μmol/g per min)
E. coli B/r + HCHO		
Expt A[a]	0	4.8, 6.6
	7	7.3
	14	7.2
	21	8.9 ± 1.3 (4)[b]
	28	9.9 ± 2.7 (5)[b]
Expt B[c]	0	13.5
	30	11.1
	90	92.1 ± 0.6 (3)[b]
E. coli ML 35		
Expt C	0	6.9
	30	5.8

[a] These results were part of the experiment shown in Table 2. TDG was present in some of the samples for 21 and 28 min of irradiation. There was no evident trend with increased levels of TDG at the levels indicated in Table 2, and all results at each particular dose have been averaged.

[b] Mean ± standard deviation (number of measurements in repeated experiments).

[c] These results are part of the experiment shown in Fig. 1.

rapidly than cells irradiated in buffer only (cf. Fig. 1 and 2). Moreover, each recorder trace for ONPG hydrolysis showed a progressive decrease in slope during the assay measurement. The observations indicated that, in addition to the inactivation by near-UV alone, some constituent in the medium was also inactivating the cells. This was confirmed by adding irradiated, CAP-containing media to unirradiated cells and examining the effect upon TMG uptake. We found that exposure of CAP to near-UV for 20 min before addition (final concentration, 100 μg/ml) to the culture inactivated TMG uptake exponentially with a half-time of approximately 10 min. This photochemical reaction is perhaps not a surprising finding since it is well known that, although CAP solutions are transparent in the visible range, they must be protected from sunlight to retain potency. At the concentration employed (100 μg/ml), CAP has an absorbance such that 68% of the light at 313 nm and 7% of the light at 365 nm would be absorbed in the experiment and might serve as a dye in photodynamic inactivation.

CAP-sensitized inactivation permitted another test of the independence of the action of near-UV on the energy supply and on the transport system itself; the results of assays performed immediately after termination of irradiation of bacterial suspensions in the presence of CAP are presented in Fig. 2. Because we were not able to prevent continuing indirect inactivation during and after exposure, the results depend not only upon the time periods required for each irradiation and, therefore, upon the fluence rate, but also on the subsequent manipulation time (including time to centrifuge the sample to the end of either assay). The nonlinearity resulting from the continuing inactivation gives the downward curvature seen in Fig. 2. Even with these difficulties, however, the results qualitatively show that both the uphill (open squares) and downhill (closed squares) transport have similar sensitivities to the near-UV in the presence of CAP. These results contrast with those shown in Fig. 1, where TMG accumulation is about twice as sensitive as ONPG hydrolysis to irradiation.

As demonstrated above, energy supply is not a significant factor in the inactivation of ONPG hydrolysis. Thus, because TMG transport and ONPG hydrolysis have the same heightened sensitivity to near-UV in the presence of CAP, it would appear that it is not the energy supply that is interrupted by the photosensitized process. These results imply, therefore, that the CAP-photosensitized process is primarily a chemical or structural alteration of the permease.

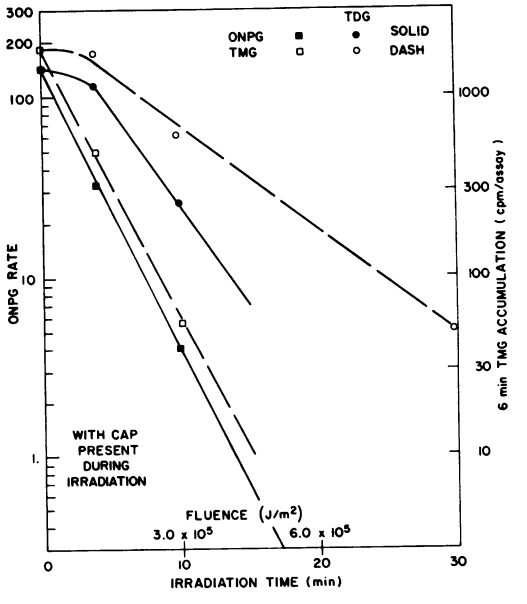

FIG. 2. *Inactivation of galactoside transport by blacklight in the presence of CAP. See legend to Fig. 1.*

A second finding shown in Fig. 2 is that transport by either measure was strongly protected when TDG was added to the irradiation medium. Confirmation of this protection of TMG transport was obtained in another experiment in which the apparent half-life was increased from 1.5 to 5.2 min by irradiation in the presence of TDG. Protection by TDG in the presence of CAP contrasts with its failure to protect the uphill assay against exposure to near-UV in the absence of CAP. These results further support the interpretation that the CAP-photosensitized process acts primarily upon the permease.

We also observed CAP-photosensitized inactivation of the transport systems for leucine and proline in addition to the galactoside permease system.

Inactivation of β-galactosidase. Table 4 shows that the cytoplasmic enzyme β-galactosidase was not inactivated at comparatively small fluences of near-UV light in the presence of CAP. Inactivation only occurred at rather large doses comparable to those producing leaky cell membranes. Minor inactivation of β-galactosidase does not invalidate the net in vivo permease measurements because the inactivation of permease occurs at much smaller doses, and internal amounts of β-galactosidase are, therefore, in even greater excess relative to the carrier as the irradiation proceeds.

DISCUSSION

To study the effects of near-UV on transport we chose the galactoside permease system because assays are available with different dependencies on metabolic energy. The steady-state plateau accumulation of [^{14}C]TMG is the assay most dependent on metabolic energy since azide, cyanide, or proton conductors readily abolish this capability. For the steady-state accumulation the permease is necessary to accumulate internal pools, but pool size is independent of the number of permeases per cell (10, 12). The least energy-dependent assay available is the in vivo hydrolysis of ONPG in the presence of CCCP. This is a difficult system to work with because of concomitant and progressive inactivation of the permease system (4). However, we were able to use it to show that the downhill assay of in vivo hydrolysis in the absence of CCCP did not become energy limited even after 60 min of illumination. That fluence fully inactivated uphill transport. This situation is parallel to the effect of azide, which abolishes uphill and only slightly inhibits downhill transport; it takes severe energy depletion procedures to block the latter process.

TABLE 4. *Effect of near-UV on β-galactosidase*

Determination	Irradiation time (min)	β-Galactosidase activity (μmol/g per min)
Expt A[a]		
Control		4,100
Near-UV	12	4,280
Expt B[b]		
Control		3,290
Near-UV	60	1,690

[a] *E. coli* ML 35 I^- Z^+ Y^- in presence of CAP plus glycerol. This dose would inactivate the permease-mediated activity of strain B/r by 98%.

[b] IPTG-induced *E. coli* B/r; no CAP present. These assays were part of the experiment shown in Fig. 1.

The results presented here show that near-UV acts on the cell to interfere with active membrane transport in at least three distinct ways.

First, near-UV interferes with the flow of metabolic energy. The capability of cells to accumulate TMG is inactivated by near-UV more rapidly than is the capability of the same suspension of cells to transport ONPG down a chemical gradient. TDG does not protect against this process nor does CAP potentiate it.

Second, near-UV also inactivates the galactoside permease system itself, as ascertained by the downhill assay with ONPG and by the effects of CCCP on that assay. When present during the irradiation, TDG protected against this kind of damage and CAP enhanced it.

Third, large fluences of near-UV light increase the passive permeability of cells via processes not mediated by galactoside permease (Table 3). It is not clear whether irradiation-produced membrane imperfections give rise to channels or pores in the membrane or to chemical changes in the membrane that lead to increased solubility of polar compounds or increased fluidity of the cytoplasmic membrane. An increase in permeability would interfere with active transport of TMG since the accumulated solute could then escape from the cell more readily. These permeability changes are nonlinear with dose and irradiation and are significant only at high fluences where the permease function would be abolished.

Although our experiments reveal that there is more than one site of action of near-UV radiation, they do not bear on how directly any of these processes follow after absorption of the electromagnetic radiation. The actual chromophores may be quinones, flavins, cytochromes, or as yet unsuspected compounds. Interaction may require molecular oxygen and proceed through singlet oxygen or ring peroxides. The

primary chromophore might be a membrane component, and one or a series of active intermediates may be involved. Although the CAP-mediated effects are obviously indirect, it may be that the other reactions also are indirect. Indeed, the final chemical action may not be either on a component of the energy production-coupling machinery or on the components of the galactosidase transport machinery, but may possibly affect membrane components that form the substratum in which these mechanisms function.

Nevertheless, protection of the permease by TDG both against the direct and CAP-photosensitized inactivation suggests one of the following alternatives; (i) TDG binding shields a vulnerable permease site against photoproducts; (ii) binding causes a conformational change that protects the site; or (iii) binding causes movement of the site to a protected portion of the membrane. TDG does not protect against the inactivation of the energy flux, as shown by our observation that TMG transport was not protected in the presence of TDG. This suggests that TDG does not bind to the energy system and may not penetrate to those parts of the membrane concerned with energy supply.

The damage produced by the irradiated CAP also is expressed entirely or almost exclusively on the permease system and not on its energy supply or coupling to that supply. This conclusion follows from the fact that both measures of transport were decreased to the same degree and both were protected by TDG (Fig. 2). Since only an infinitesimal amount of the CAP can be within the cytoplasmic membrane, this selectivity towards the permease system may become understandable. The primary interaction of CAP with the radiant energy takes place largely outside of cytoplasmic membrane, and the permease carrier must necessarily come in contact with the aqueous interfaces of the cytoplasmic membranes.

Finally, we note that the protection by TDG of permease against near-UV appears similar to the protection of the same galactoside permease system by TDG against *N*-ethyl maleimide attack at critical sulfhydryl group site II of the permease M protein (3, 6). Whether or not near-UV irradiation results in the inactivation of the permease product at the same site, the techniques used in this report may provide a probe for further study of permease structure and function in situ.

ACKNOWLEDGMENTS

We wish to thank M. C. Escher for stimulating ideas. This work was supported by the U. S. Energy Research and Development Administration.

LITERATURE CITED

1. **Boniface, J., and A. L. Koch.** 1967. The interaction between permeases as a tool to find their relationship on the membrane. Biochim. Biophys. Acta 135:756-770.
2. **Brock, T. D., and M. L. Brock.** 1959. Effect of novobiocin on permeability of *Escherichia coli*. Arch. Biochem. Biophys. 85:176-185.
3. **Carter, J. R., Jr., C. F. Fox, and E. P. Kennedy.** 1968. Interaction of sugars with the membrane protein component of the lactose transport system of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. 61:725-732.
4. **Cecchini, G., and A. L. Koch.** 1975. Effect of uncouplers on "downhill" β-galactoside transport in energy-depleted cells of *Escherichia coli*. J. Bacteriol. 123:187-195.
5. **D'Aoust, J. Y., J. Giroux, L. R. Barran, H. Schneider, and W. G. Martin.** 1974. Some effects of visible light on *Escherichia coli*. J. Bacteriol. 120:799-804.
6. **Fox, C. F., and E. P. Kennedy.** 1965. Specific labeling and partial purification of the M protein, a component of the β-galactoside transport system of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. 54:891-899.
7. **Jagger, J.** 1972. Growth delay, and photoprotection induced by near-ultraviolet light, p. 383-341. *In* U. Gallo and L. Santamaria (ed.), Research progress in organic-biological and medicinal chemistry, vol. 3. North-Holland Publishing Company, Amsterdam.
8. **Kashket, E. R., and A. F. Brodie.** 1962. Effects of near-ultraviolet irradiation on growth and oxidative metabolism of bacteria. J. Bacteriol. 83:1094-1100.
9. **Kashket, E. R., and A. F. Brodie.** 1963. Oxidative phosphorylation in fractionated bacterial systems. X. Different roles for the natural quinones of *Escherichia coli* W in oxidative metabolism. J. Biol. Chem. 238:2564-2570.
10. **Kepes, A., and G. N. Cohen.** 1962. Permeation, p. 179-221. *In* I. C. Gunsalus and R. Y. Stanier (ed.), The bacteria, vol. 4. Academic Press Inc., New York.
11. **Koch, A. L.** 1963. The inactivation of the transport mechanism for β-galactosidase of *Escherichia coli* under various physiological conditions. Ann. N.Y. Acad. Sci. 102:602-620.
12. **Koch, A. L.** 1964. The role of permease in transport. Biochim. Biophys. Acta 79:177-200.
13. **Koch, A. L.** 1971. Energy expenditure is obligatory for the downhill transport of galactosides. J. Mol. Biol. 59:447-459.
14. **Lo, T. C. Y., M. K. Rayman, and B. D. Sanwal.** 1974. Transport succinate in *Escherichia coli*. III. Biochemical and genetic studies of mechanism of transport in membrane vesicles. Car:. J. Biochem. 52:854-866.
15. **MacLeod, R. A., P. Thurman, and H. J. Rogers.** 1973. Comparative transport activity of intact cells, membrane vesicles, and mesosomes of *Bacillus licheniformis*. J. Bacteriol. 113:329-340.
16. **Peak, M. J., and J. G. Peak.** 1973. Protection by histidine from inactivation of DNA transforming activity by near-ultraviolet light (365 nm) compared with far-ultraviolet light (254 nm). Photochem. Photobiol. 18:525-527.
17. **Miller, J. H.** 1972. Experiments in molecular genetics, p. 431. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
18. **Robbins, J. C., and D. L. Oxender.** 1973. Transport systems for alanine, serine, and glycine in *Escherichia coli* K-12. J. Bacteriol. 116:12-18.
19. **Werben, H., B. D. Lakchaura, and J. Jagger.** 1974. Near-ultraviolet modification of *Escherichia coli* B ubiquinone *in vivo* and *in vitro*. Photochem. Photobiol. 19:321-328.
20. **West, I., and P. Mitchell.** 1972. Proton-coupled β-galactoside translocation in non-metabolising *Escherichia coli*. J. Bioenerg. 3:445-462.