The Effect of Ultraviolet Light on the Hydrogenase of *Proteus vulgaris**

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ABSTRACT: The hydrogenase activity of *Proteus vulgaris* can be activated by irradiation with light of wavelength 2537 Å. This activation is dependent on the light intensity and persists after the irradiation is terminated and the gas phase is replaced with a new sample of gas. The hydrogenase remains activated after exposure to air. The activation also occurs in the presence of carbon monoxide and is due to activation of the residual active enzyme and not to a reversal of the CO inhibition. Prolonged irradiation with ultraviolet light leads to inactivation of the hydrogenase. With the light intensities employed, visible light had a greater activating effect than ultraviolet light. A short-term irradiation with visible or ultraviolet light activates hydrogenase if the enzyme is in the active deoxygenated form during irradiation.

The inhibition of the enzyme hydrogenase by carbon monoxide has been observed by many investigators, but with the exception of Hoberman and Rittenberg (1943) and Kempner and Kubowitz (1933), all have failed to demonstrate its reversibility by light. In 1962, we demonstrated that the activity of the hydrogenase of *Proteus vulgaris* which is inhibited by carbon monoxide could be substantially increased by exposing the inhibited enzyme to strong visible light sources (Purec *et al.*, 1962). We interpreted this finding to be due to the reversal of the CO inhibition as is the case with many enzymes. We had also observed that the light effect persists even when the illumination is withdrawn. The CO inhibition of the hydrogenase of *Desulfovibrio desulfuricans* has been reported (Sadana and Rittenberg, 1963) to be reversed by visible light and the reversal disappeared when the illumination was withdrawn.

Recently, we (Purec and Krasna, 1967) reinvestigated the light activation of the hydrogenase of *P. vulgaris* in the presence of carbon monoxide and found that visible light strongly activates this hydrogenase even in the absence of carbon monoxide. It was shown that visible light does not reverse the CO inhibition but stimulates the activity of the residual active enzyme. This activation of hydrogenase in the absence of CO persisted after the light source was removed and when the gas phase was replaced by a new sample of the same gas. It also persisted when the cells were exposed to air after being activated by light in a hydrogen atmosphere.

In preliminary experiments to determine the action spectrum of the light activation, it was observed that although there appeared to be activation throughout the visible region, light of shorter wavelengths seemed to have a greater effect than longer wavelengths. It therefore was of interest to study the effect of ultraviolet light of wavelength 2537 Å on the activation of the hydrogenase of *P. vulgaris*. As reported below, light of this wavelength activates hydrogenase, but prolonged exposure to ultraviolet light causes inactivation of the enzyme.

Experimental Section

The experimental details were similar to those described previously (Purec and Krasna, 1967; Purec *et al.*, 1962). Cells of *P. vulgaris* were grown on complex media (Krasna and Rittenberg, 1954) and were used as the source of hydrogenase. The deuterium-exchange assay was carried out with 10% D_2O and the tritium-exchange assay (Anand and Krasna, 1965) was carried out with 1 mc/ml of THO. All experiments were carried out in 0.15 M phosphate buffer, pH 6.7. The rate of exchange was always measured in the range where the rate was proportional to enzyme concentration (Krasna and Rittenberg, 1957).

The experimental arrangement for using a visible light source has already been described (Purec and Krasna, 1967). It gave a total intensity in the visible region of 20 w/ft². The total intensity between 3500 and 4500 Å was about 1.8 w/ft² or an average of 3.9×10^{-7} einstein/cm² per min at 4000 Å. (Preliminary experiments suggest that light between 3500 and 4500 Å is responsible for about 50% of the visible-light effect.) The ultraviolet light source was one or two 15-w General Electric germicidal lamps G15T8 kept 13 cm from the reaction flask which was constructed of quartz. Each lamp has an output of about 3 w at 2537 Å and gives an intensity of 1 w/ft² (1.4×10^{-7} einstein/cm² per min) at the reaction flask. The emission spectrum of the lamp was deter-

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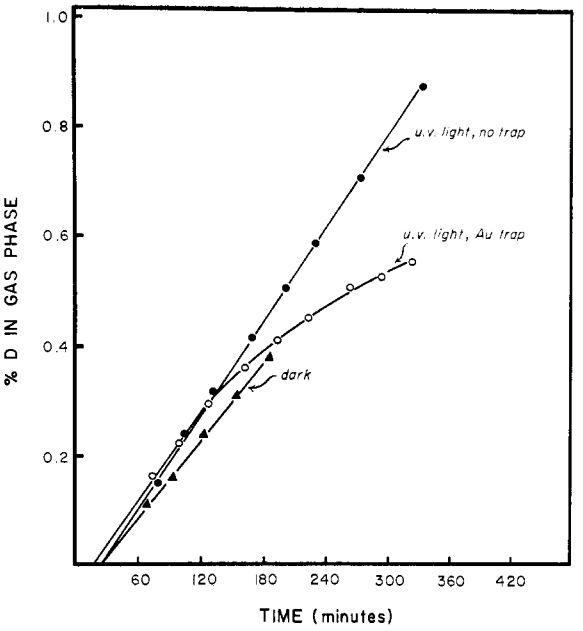

FIGURE 1: Effect of ultraviolet light on hydrogenase activity in the presence of CO in the presence and absence of mercury vapor. One of the flasks was connected to the vacuum manifold through a gold trap in order to adsorb all the mercury vapor, and the other two flasks were connected directly to the vacuum manifold. The flasks in the light were illuminated with two 15-w germicidal lamps. (▲) Dark, no trap; rate = 0.142% D/hr. (●) Light, no trap; rate = 0.183% D/hr. (○) Light, gold trap; initial rate = 0.183% D/hr and final rate = 0.070% D/hr.

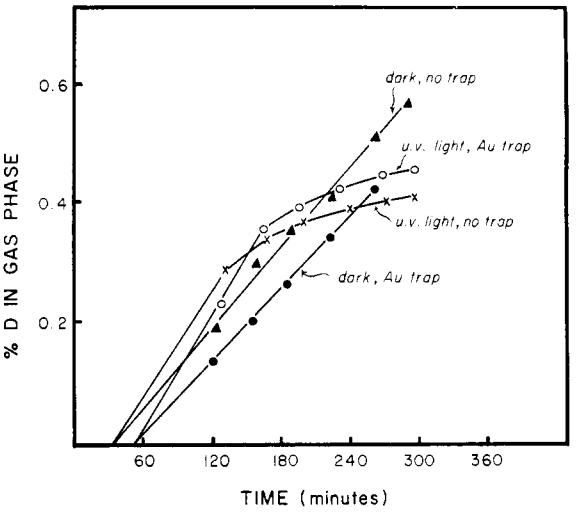

FIGURE 2: Effect of ultraviolet light on hydrogenase activity in the absence of CO in the presence and absence of mercury vapor. Two of the flasks were connected to the vacuum manifold through a gold trap in order to adsorb all the mercury vapor, and the other two flasks were connected directly to the vacuum manifold. The flasks in the light were illuminated with two 15-w germicidal lamps. (●) Dark, Au trap; (▲) dark, no trap; (○) light, Au trap; (×) light, no trap.

mined with a Cary Model 14 spectrophotometer and gave the following relative emissions: 2537 Å, 58%; 4010 Å, 5%; 4050 Å, 5%; 4360 Å, 22%; 5461 Å, 8%; and 3130, 3641, 5810, and 5825 Å, 0.5% each. When lower light intensities were desired, neutral density filters were placed in the light path in front of the reaction flask.

The activation of hydrogenase with this ultraviolet lamp described in the Results section is due to the 2537-Å component of the emitted light and not to any of the visible components. With a Pyrex reaction flask which does not transmit below 3000 Å, there was no activation of hydrogenase. There was also no activation with a filter of 0.5% KI plus 0.2% Na_2SO_3 which does not transmit any light below 2600 Å but transmits completely above this wavelength. There was normal activation using a filter of 0.005% KI plus 0.002% Na_2SO_3 which transmits above 2400 Å.

Because of the intense scattering from whole cell suspensions, the absorption spectrum was measured on cell-free extracts. With 0.04 ml of extract in 5 ml of buffer (corresponding to the quantity of cells used in the experiments described) there was an absorption peak at 2537 Å with an absorbance of 1.34. There was comparatively little absorption in the visible region; at 3200 Å the absorbance was 0.08 which gradually decreased to 0.004 at 7000 Å. Between 3500 and 4500 Å the absorbance averaged 0.05. It must be stressed that these absorbances are for a crude cell extract and the contribution of hydrogenase to the absorbance is probably extremely small. The extract contains over 20% nucleic acid and this alone may be responsible for over 90% of the 2537-Å absorbance.

When a gas mixture of 25% CO–75% H_2 was irradiated with ultraviolet light, it was observed that the gas pressure decreased about 10% after a 2-hr irradiation making it impossible to use the tritium assay which depends on the removal of stoichiometric aliquots. This decrease in pressure was shown to be due to the formation of formaldehyde from CO and H_2 catalyzed by mercury atoms photosensitized by irradiation at 2537 Å. Formaldehyde was determined by the use of chromotropic acid (Neidig and Hess, 1952; Altshuller *et al.*, 1961) and by preparation of the crystalline dimedon derivative. The formation of formaldehyde was not observed in the dark or in visible light. The formation of formaldehyde by irradiation of a mixture of H_2 and CO with light of 2537-Å wavelength in the presence of mercury vapor has been reported previously (Taylor and Marshall, 1925; Taylor, 1926) and was shown to be due to the activation of H_2 and not CO.

Since the vacuum manifold employs a mercury-diffusion pump and mercury manometers, all the gases are saturated with mercury vapor. To remove mercury vapor from the gases, the reaction flasks were connected to the vacuum manifold through a glass U tube (24 cm

long × 0.5 cm i.d.) containing 16 grains of pure gold foil. When the mercury vapor was removed in this manner, irradiation of $CO-H_2$ mixtures with ultraviolet light did not cause a decrease in pressure and did not produce formaldehyde.

Results

Effect of Ultraviolet Light in the Presence of CO. The effect of ultraviolet light on hydrogenase in the presence of carbon monoxide in the presence and absence of mercury vapor is shown in Figure 1. The rate in the dark represents a 54% inhibition of the rate obtained in 25% N_2–75% H_2 and was the same in the presence or absence of mercury vapor. In the presence of mercury vapor, ultraviolet light caused a 30% increase in exchange activity while in the absence of mercury vapor ultraviolet light had a dual effect. Initially, there was the same activation as in the presence of mercury vapor, but as illumination continued, the rate of reaction decreased, the final rate being less than 50% of the rate in the dark.

The activation of hydrogenase in the presence of carbon monoxide by ultraviolet light is undoubtedly due to an actual activation of hydrogenase since identical initial rates were obtained whether mercury vapor was present or not. The inactivation of the hydrogenase by prolonged ultraviolet irradiation in the absence of mercury is undoubtedly due to photochemical inactivation of the enzyme (see Discussion). The failure to observe this inactivation in the presence of mercury vapor is not due to the presence of formaldehyde formed nonenzymatically since addition of formaldehyde in the absence of mercury vapor did not prevent the inactivation. We cannot at present offer any unequivocal explanation for this observation.

Effect of Ultraviolet Light in the Absence of Carbon Monoxide. Since it was recently demonstrated (Purec and Krasna, 1967) that the activation of the hydrogenase of *P. vulgaris* by visible light was not dependent on the presence of CO, it was of importance to determine whether this was also the case for ultraviolet activation. Figure 2 shows the effect of ultraviolet light on hydrogenase activity in the absence of CO in the presence and absence of mercury vapor. It is clear that the presence or absence of mercury vapor does not affect the activity of hydrogenase in the dark. Ultraviolet light initially activates the hydrogenase 30% and there is a progressive inactivation as illumination continues. In this case, with no CO present, there is no difference whether mercury vapor is present or not. Addition of formaldehyde had no effect on the activation or inactivation.

In order to determine whether the activation of hydrogenase by ultraviolet light is quantitatively the same in the presence and absence of CO, *i.e.*, whether there is *any* CO reversal in ultraviolet light, the experiment was performed under conditions permitting the inactivation to be deferred for a reasonable period of time, sufficient to obtain reliable rate measurements on the activation. This was accomplished by decreasing the ultraviolet intensity (see below) by using one lamp instead of two. The results of such an experiment in the absence of mercury vapor are shown in Table I. Since 25% CO inhibits the hydrogenase of *P. vulgaris* 50%, twice as many cells were added to the flasks in $CO-H_2$ as in N_2-H_2 in order to obtain comparable rates. It can be seen that there is no difference in the ultraviolet activation in the presence or absence of CO. Thus, the effect of ultraviolet light on the hydrogenase of *P. vulgaris* is the same as of visible light, *i.e.*, an activation of active hydrogenase and no reversal of CO inhibition. The following experiments were, therefore, all performed in a gas phase of H_2 and, since mercury had no effect on the activation or inactivation in H_2, they were all done without removing the mercury vapor.

TABLE I: Effect of Ultraviolet Light on the Hydrogenase Activity of *P. vulgaris* in the Presence and Absence of Carbon Monoxide.[a]

Gas Phase	Condition	Rate (mv/hr)	% Activation
25% CO–75% H_2	Dark	2.80	
	Ultraviolet	3.74	34
25% N_2–75% H_2	Dark	2.75	
	Ultraviolet	3.60	31

[a] The flasks containing the cell suspensions in a final concentration of THO of 1 mc/ml were evacuated and filled with the indicated gas mixtures. The flasks with N_2-H_2 contained 0.12 ml of the cell suspension and the flasks with $CO-H_2$ contained 0.24 ml of the cell suspension. All flasks were connected to the vacuum manifold through a gold trap to absorb the mercury vapor. The flasks in the light were irradiated with a single 15-w germicidal lamp.

Effect of Ultraviolet Duration and Intensity. Since ultraviolet light initially causes an activation of hydrogenase, it seemed reasonable that exposure to short periods of irradiation might cause only activation and not inhibition. Cells were exposed to ultraviolet light of high intensity for the first 30 min, 60 min, and continuously. Exposure for 30 min gave 24% activation while exposure for 60 min gave 51% activation. There was no inactivation with these exposure times. Continuous exposure to ultraviolet light, on the other hand, gave an initial activation of 65% followed by inactivation. When the effect of continuous ultraviolet irradiation of different intensities was studied it was found that the activation increased with increasing ultraviolet intensity and inactivation was only observed with the maximum light intensity after 3 hr of irradiation.

Comparison of Visible and Ultraviolet Light. In Figure 3 the effect of visible and ultraviolet light on hydrogenase is compared along with the combined effect of both light sources. It is clear that with the light intensities employed the activation by visible light is about twice

TABLE II: Effect of the Removal of Light and Change of Gas Phase on Ultraviolet Activation of Hydrogenase.[a]

Phase 1		Phase 2		Phase 3	
Condition	Rate (mv/hr)	Condition	Rate (mv/hr)	Condition	Rate (mv/hr)
Dark	2.81	Dark	2.60	Dark	2.60
Ultraviolet light	3.64	Ultraviolet light	4.02	Dark	3.43
Ultraviolet light	4.03	Dark	3.99	Dark	3.80

[a] The ultraviolet source was one 15-w germicidal lamp. The flasks were kept in the condition described in phase 1 for 3 hr at which time all flasks were evacuated and filled with a new sample of H_2 gas and placed in the light or dark as described in phase 2. Phase 2 was continued for 3 hr at which time the flasks were opened to the air and kept for 24 hr in the refrigerator in this condition. Then sodium hydrosulfite (5 mg/ml) was added to each flask before evacuation and the flasks were evacuated and filled with H_2 and shaken in the dark. The rates obtained after being exposed to air are listed under phase 3.

that obtained with ultraviolet, while the activation with both light sources together is initially about the same as with visible alone. Ultraviolet eventually leads to inactivation even in the presence of visible light. The greater activation by both light sources together compared with ultraviolet alone probably stems from a greater activating effect of visible light.

Effect of Removal of Light Source and Change of Gas Phase. In the previous investigation (Purec and Krasna, 1967) it was shown that the activation by visible light continues after the illumination is terminated whether or not the gas phase is changed. If the activation by ultraviolet light affects the enzyme in the same manner as visible light, then the activation should persist after the ultraviolet illumination is terminated and the gas phase is replaced with a new sample of H_2. That this is the case is shown by the data in Table II. During phase 2, when the original gas sample was removed and replaced with a new sample of H_2, the original activation by ultraviolet light persisted whether the light source was present or not. Identical results were obtained when the gas was not changed between phase 1 and phase 2. The activation persisted even after the cells were exposed to air for 24 hr (phase 3).

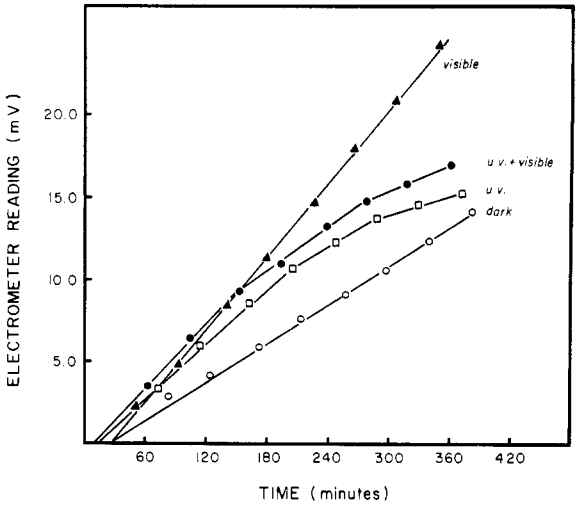

FIGURE 3: Effect of visible and ultraviolet light on the hydrogenase of *P. vulgaris*. One flask was kept in the dark, one irradiated with a visible light source, one irradiated with two 15-w germicidal lamps, and one irradiated with both light sources at the same time. (○) Dark; (□) ultraviolet light; (▲) visible light; (●) ultraviolet light and visible light.

To determine whether the ultraviolet inactivation of hydrogenase observed after prolonged exposure could be reversed by removing the illumination, experiments similar to those described in Table II were carried out under conditions where inactivation took place, *i.e.*, by intense irradiation for prolonged periods of time. Removal of the light source did not reverse the inactivation. In fact, the degree of inactivation increased on incubation in the dark even after the cells were exposed to air. This postirradiation inactivation may suggest that an inactivating agent is produced during irradiation which continues to inactivate the enzyme after the light is withdrawn.

Effect of Short-Term Irradiation under Different Conditions. We have shown here that a relatively short exposure to ultraviolet light in H_2 suffices to activate hydrogenase. It was of interest to determine whether this is also the case for visible light and whether the hydrogenase must be in the active deoxygenated form in order for the activity to be increased by light. The data in Table III summarize the results of such a study. A 1-hr irradiation with visible or ultraviolet light in a H_2 atmosphere increased the activity of hydrogenase, visible light having a greater effect than ultraviolet light. When the hydrogenase is in the inactive form (no preactivation), irradiation in N_2 or CO had very little effect, while irradiation in air gave 30% inactivation. When, however, the hydrogenase was preactivated by prior incubation in H_2, short-term irradiation in N_2 or CO increased the activity, while there was very little effect on irradia-

TABLE III: Effect of Short-Term Irradiation under Different Conditions.[a]

Gas Phase during Irradiation	% Activation			
	No Preactivation		Preactivation	
	Visible	Ultraviolet	Visible	Ultraviolet
H_2	60	40	70	40
Air	−35	−30	8	10
N_2	7	12	60	40
CO	8	12	35	35

[a] The flasks containing the cells were evacuated and filled with the gases indicated and were irradiated with visible light or ultraviolet light for 1 hr. When preactivation was desired, the flasks were first filled with H_2 and shaken for 2 hr in the dark before filling with the appropriate gas. After the irradiation was completed, the flasks were opened to the air momentarily, sodium hydrosulfite was added (5 mg/ml), and the flasks were evacuated, filled with H_2, and shaken in the dark where the rates were measured.

tion in air. After exposure to air, hydrogenase becomes oxygenated and it is inexplicable why there should be a difference if the hydrogenase is preactivated or not. The finding that irradiation in N_2 or CO caused activation only in preactivated cells suggests that the activation takes place on the active deoxygenated form of the enzyme and is not directly influenced by N_2 or CO.

Discussion

The activation of the hydrogenase of *P. vulgaris* by visible light previously reported (Purec and Krasna, 1967) has now been shown to occur with ultraviolet light of wavelength 2537 Å as well. With the light intensities employed, visible light had a greater activating effect than ultraviolet light but it is possible that this may in part be due to partial inactivation by ultraviolet light. In order to quantitatively compare the activation caused by visible and ultraviolet light, one should calculate the quantum yields at the different wavelengths. However, since a pure enzyme preparation was not available, it is impossible to calculate any meaningful quantum yield. The extinction coefficient of the enzyme at different wavelengths and the enzyme content of the cells or extract is not known. We also do not know what per cent of the measured absorption is due to the hydrogenase and which wavelengths in the visible are responsible for the activation. The only comparison that can be made is with the incident intensities. An incident intensity of 2.8×10^{-7} einstein/cm^2 per min at 2537 Å gives about the same activation (50%) as 3.9×10^{-7} einstein/cm^2 per min between 3500 and 4500 Å (see Experimental Section).

A short-term irradiation with either light source suffices to activate hydrogenase if the hydrogenase is in the active deoxygenated form. The mechanism of this light activation is not known but it is of interest that it takes place at such widely different wavelengths. Preliminary experiments indicate that light activation is also observed with cell-free extracts and would suggest therefore that permeability is not a factor in the activation. The activation may be due to the induction of a conformational change in the enzyme which increases its catalytic activity. It may also be due to the destruction of an unknown inhibitor normally present.

Ultraviolet light leads to inactivation of hydrogenase on prolonged irradiation. This, undoubtedly is due to irreversible denaturation and degradation of the enzyme by light of wavelength 2537 Å. It is well known (McLaren and Shugar, 1964; Setlow, 1957; Errera, 1953) that light of this wavelength causes irreversible denaturation of proteins and inactivation of many enzymes.

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