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Photoactivation of Nitrile Hydratase in Corynebacterium sp. N-774

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Irradiation of cells or extracts of Corynebacterium sp. N-774 with light increased nitrile hydratase activity. The effective wavelength was about 370 nm, i.e., the near-ultraviolet region. When stored at 0 °C in the dark, the enzyme activity of cells gradually decreased, and most of the lost activity could be restored by irradiation with light.

Biological photochemical phenomena, such as photosynthesis, phototropism, phototaxis, photoperiodism, photoregulation of germination of efflorescence, protontransport in Halobacterium and visual excitation are well known.

Concerning with the microbial transformation of nitrile compounds, we have been studying acrylamide production from acrylonitrile using Corynebacterium sp. N-774,[1] which exhibits nitrile hydratase activity. During this work, we found a new photochemical phenomenon, that is, the nitrile hydratase of this bacterium was greatly enhanced by light irradiation. Similar stimulation of enzyme activity by light has been reported, i.e., urocanase activity of cells or extracts of Pseudomonas putida[2] was increased by breaf exposure to near-ultraviolet light radiation. To clarify this phenomenon and the mechanism involved in more detail, we performed some additional experiments. This paper deals with the photoactivation of the enzyme in cells and cell-free extract, the changes in the enzyme activity under light and dark conditions, and the effective wavelength for the enzyme activity.

Enzyme activity under light and dark conditions. To clarify the phenomenon of photoactivation of the enzyme as to either the cell or enzyme level, particularly with strain N-774 or other strains, the enzyme activity was examined under light and dark conditions. Table 1 shows the enzyme activities of cells and cell-free extract of strain N-774 and Pseudomonas chlororaphis B23,[3] which exhibit nitrile hydratase activity. As shown in Table 1, the photoactivation was found to occur immediately on irradiation with light in cells and cell-free extract of strain N-774, and in the case of cell-free extract, the enzyme activity, once enhanced, showed substantially no differences under light and dark conditions, whereas the enzyme activity of Ps. chlororaphis B23 was not entirely affected by light

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Table 1. Nitrile Hydratase Activity under Light and Dark Conditions

Cells and cell-free extract	Specific activity (units/mg dry cell or protein)	
	Light	Dark
Corynebacterium sp. N-774		
Cells	38.5	1.2
Cell-free extract A	42.8	42.8
Cell-free extract B	42.0	2.0
Ps. chlororaphis B23		
Cells	55.1	55.0

Medium and Cultivation: Using MY [a] medium(strain N-774) and SMM [b] medium(Ps. chlororaphis B23), cultivation was carried out for 48 h at 30 °C or 25 °C, respectively, with shaking in the dark.
Cell-free extract of strain N-774: Cells cultivated in MY medium were washed with 0.05 M potassium phosphate buffer(pH 7.7) and then disrupted with a French Press at 1300 kg/cm^2. Cells and debris were removed by centrifugation at 15000 xg for 30 min and the resultant supernatant was used as cell-free extract A. In the preparation of cell-free extract B, all the procedures were carried out in the dark.
Measurement of enzyme activity: The reaction mixtures (10 ml) contained 4.72 mM acrylonitrile, 0.5 mM potassium phosphate buffer(pH 7.7) and an appropriate amount of the enzyme solution or cells. The reaction was carried out at 10 °C for 10 min. Acrylamide produced was measured by gas chromatography; glass column packed with Porapak PS, 210 °C(column), 240 °C(detector), 40 cm^3/min(N_2 flow rate), flame ionization detector.
Light activity: The enzyme solution was irradiated with light from a 100W EYE-LAMP manufactured by Iwasaki Denki (at a distance of 50 cm) at 0 °C for 1 h prior to the reaction and the irradiation with light was also carried out similarly during reaction.
Dark activity: The enzyme activity was measured as described above in the dark.
a): glucose 1.0%(w/v), polypeptone 0.5%, yeast extract 0.3% and malt extract 0.3%(pH 7.2).
b): sucrose 1.0%(w/v), soybean protein hydrolyzate 0.3%, methacrylamide 0.8%, KH_2PO_4 0.05%, K_2HPO_4 0.05%, $MgSO_4 \cdot 7H_2O$ 0.05% and $FeSO_4 \cdot 7H_2O$ 0.001%(pH 7.3).

irradiation. Therefore, the nitrile hydratase enzymes in strain N-774 and Ps. chlororaphis B23 seem to be different. Figure 1 shows the effect of the duration of irradiation on the activation of the enzyme in extracts from cells of strain N-774. The nitrile hydratase activity increased 4.7-fold in one min, and 21-fold in 20 min. No time lag in the activation was detected. Control vials kept in the dark showed no increase in nitrile hydratase activity during the experiment.

Expression of enzyme activity on incubation in light and dark conditions. The enzyme activities of resting cells and cell-free extract cultured and prepared,

respectively, under the dark conditions were examined. As shown in Fig. 2, in the light, a decrease in enzyme activity was not observed in either cells or cell-free extract, whereas in the dark, the activity gradually decreased in resting cells, reaching less than one-tenth of the initial activity after 30 h incubation. The enzyme activity in the cells having decreased during the dark incubation was restored to the starting level by light irradiation. And with the cell-free extract incubated in the light, only a little loss of enzyme activity was observed. This inactivation constituted an irreversible loss of activity due to denaturation. Thus, the nitrile hydratase seems to be extremely stable in the dark.

Effect of the wavelength on the enzyme activity. Using cell-free extract prepared in the dark as described above, the enzyme activity was measured in the dark after irradiation with the same light intensity at various wavelengths. As shown in Table 2, it was found that the light in the near-ultraviolet region, i.e., a wavelength of about 370 nm, was the most effective.

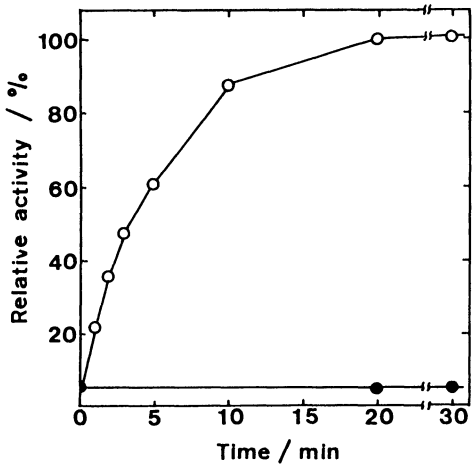

Fig. 1. Effect of irradiation time on nitrile hydratase activity of cell-free extract. Cell-free extract was irradiated at the light intensity of 20 μE/m^2·s (about 3000 lux, 100W EYE-LAMP Iwasaki Denki). The assay system, and the preparation of cell-free extract from strain N-774 are described in Table 1. Open symbols, irradiated; closed symbols, not irradiated.

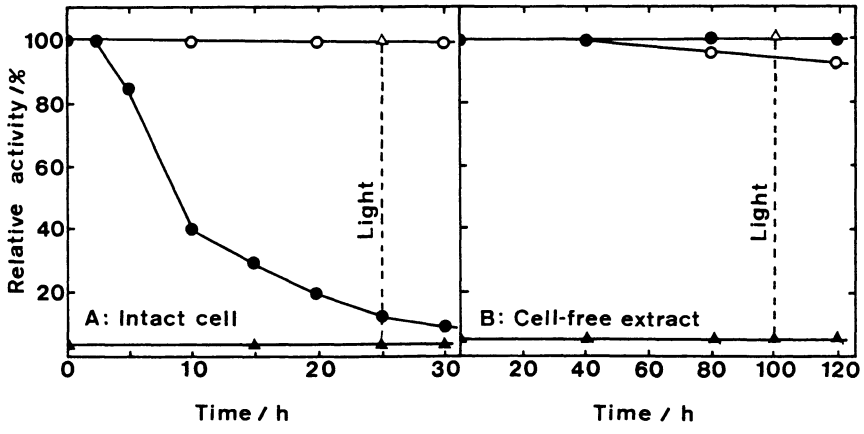

Fig. 2. Expression of nitrile hydratase activity under light and dark conditions. Cells and cell-free extract were incubated at 0 °C under the irradiation conditions given in Fig. 1. The assay system, and the preparation of cells and cell-free extract from strain N-774 are described in Table 1. Symbols: (○), enzyme activity incubating in the light. (●), enzyme activity incubating in the dark, after light irradiation. (▲), enzyme activity incubating in the dark. Cells and cell-free extract were prepared in the dark.

Table 2. Effect of the Wavelength on the Enzyme Activity

Wavelength range (nm)	Specific activity (units/mg protein)
> 660	14.9
> 580	14.4
> 480	20.6
> 450	24.1
> 370	42.0
> 340	29.3
> 280	24.5
> 220	23.6
control(dark)	2.2

Each wavelength was obtained by cutting off shorter wavelengths with color glass filters. The light irradiation was carried out for 3 min at 4 °C with the light intensity being controlled at 50.2 $\mu E/m^2 \cdot s$. (400W YOKO-LAMP Toshiba) Enzyme activity measurements were carried out under dark conditions. The preparation of cell-free extract from strain N-774 is described in Table 1.

We have found the phenomenon of photoactivation of bacterial nitrile hydratase, which participates in the hydration of aliphatic nitriles. Further studies using the purified enzyme are needed to determine the mechanism involved in this photoactivation of nitrile hydratase. Studies along these lines are currently in progress in our laboratory.

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