PHOTOINACTIVATION AND PHOTOREACTIVATION OF CONSTITUTIVE AND ADAPTIVE RESPIRATORY SYSTEMS OF AZOTOBACTER

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Acetate grown cells of Azotobacter, like sucrose grown cells (Stone and Wilson, 1952), are unadapted to the oxidation of succinic acid although possessing an enzyme system capable of oxidizing succinate. Hence the adaptation of these cells to succinic acid may be termed a functional change in the physiology of the organism. In contrast, glucose grown cells of Saccharomyces cerevisiae are unadapted to the fermentation of galactose and do not possess an enzyme system capable of fermenting this sugar. Adaptation to galactose fermentation corresponds with the appearance within the yeast cell of an apoenzyme capable of fermenting galactose (Spiegelman, 1950). The adaptation of glucose grown cells of Saccharomyces cerevisiae to galactose represents, therefore, an organic change in the enzyme component of the cell.

Functional and organic adaptation processes may be inhibited by doses of ultraviolet light, which have but a negligible effect on the activity of constitutive and adaptive respiratory enzymes (Stephenson and Yudkin, 1936; Barrett et al., 1953). The inhibition of organic adaptive processes by ultraviolet light has been shown to be abolished in yeast (Swenson and Giese, 1950) by exposing the ultraviolet irradiated cells to visible light (Kelner, 1949).

This study deals with the inhibitory effect of ultraviolet light on adaptive and constitutive respiratory systems of a bacterium and the reversal of this effect by visible light.

EXPERIMENTAL METHODS

Cells of Azotobacter agile strain A 4.4 were grown in 400 ml amounts in 1,000 ml Erlenmeyer flasks on a rotary shaker for 24 hours at 30 °C on an acetate salt medium described by Karlsson and Barker (1948). The harvested cells were washed twice with 50 ml each 0.03 M potassium phosphate buffer pH 7.0 made to 0.02 per cent MgSO₄. Twenty ml of cell suspensions containing approximately 9 x 10⁹ cells per ml were placed in a petri dish of 9 cm inside diameter, 25 cm from a 15 watt G. E. germicidal lamp, and stirred during the irradiation with a magnetic stirrer. Immediately following ultraviolet irradiation, 5.0 ml samples were withdrawn and maintained in darkness for respiratory studies. Petri dishes containing the 15 ml of the ultraviolet irradiated suspensions were then placed on a white background 10 cm distant from two 15 watt G. E. day light lamps and illuminated without stirring for 30 minutes at approximately 28 °C. Respiratory determinations on inactivated and reactivated cells were made not later than 45 minutes after inactivation.

Respiratory activity was determined using the usual Warburg techniques (Umbrat et al., 1951). The oxygen uptake of unirradiated, inactivated, and photoreactivated cells was followed at 30 °C. Warburg flask contents were as follows: 0.5 ml of cells (= 0.025 mg cell N); 0.2 ml substrate, containing either 1 x 10⁻⁴ moles of acetic acid or 5.7 x 10⁻⁹ moles of succinic acid; 2.3 ml of 0.03 M potassium phosphate buffer at pH 7.0, and 0.15 ml of 20 per cent KOH, in the center well. All solutions composing the reaction mixture were made to 0.02 per cent MgSO₄ (Goucher and Kocholaty, 1954).

RESULTS

Data presented in figure 1 show the time course of acetate oxidation by an unirradiated, irradiated, and a photoreactivated constitutive respiratory system. It is interesting to note that the capacity of the irradiated cell to oxidize acetate at a given time as expressed by the oxygen uptake values is seemingly related both to the magnitude of the ultraviolet dose to which it is exposed and to the quantity of acetate oxidized.

It is observed that the ultraviolet inactivated constitutive respiratory system involved in acetate oxidation is subject to photoreactivation.
Figure 2. Oxidation of succinate by Azotobacter agile strain A 4.4 under varying conditions of irradiation.

Conditions similar to figure 1, except that the substrate was 0.2 ml succinic acid (= 5.7 X 10^-6 moles).

DISCUSSION

The adaptation of acetate grown cells of Azotobacter to succinic acid is inhibited by azide (Goucher, 1951; Williams and Wilson, 1954) and accelerated in the presence of acetate (Williams and Wilson, 1954; Karlson, 1944). These observations may suggest that the operation of an energy yielding process is required for the adaptation to succinic acid. In addition to an energy requirement the mechanism of suc-
cinate adaptation appears to possess regulatory components which absorb in the 2537 A region. It may be speculated that these components are purines and pyrimidines, and that their chemical integrity is decisive both in affecting succinate adaptation and in the maintenance of constitutive enzyme activity. Studies, now in progress, of the extracts of ultraviolet inactivated cells obtained by sonic vibration have shown that the succinate oxidation is not affected by ultraviolet doses which inhibit the oxidation of succinate by whole bacterial cells. The absorption of ultraviolet light appears to affect the organization of the catalytic components of the cell, or cell permeability rather than the oxidative catalyst. The substances absorbing inactivating light may alter those chemical compounds and physiological mechanisms which determine whether or not the intact molecules of the respiratory catalyst are functional within the cell.

The labile state of cellular organization is emphasized by the observation that the constitutive ability of the cell to oxidize acetate is dependent possibly on factors other than the enzyme involved in the degradation of this acid. If ultraviolet inhibits acetate oxidation by making the cell impermeable to acetate, then it may be hypothesized that the permeability status of the organism is dependent on the identity of those components which absorb the inactivating radiation.

The adaptive oxidative patterns in Azotobacter have been a subject of sustained interest. This interest has been engendered not only by the ability of the organism to utilize atmospheric nitrogen for growth but also by the unusual oxidative capacity of both whole cells (Lineweaver, 1933) and cell-free extracts (Stone and Wilson, 1952). The observation of the photo-reactivation of these bacterial respiratory systems represents a new parameter in the study of a functional adaptation process, and photoinactivation and reactivation phenomena.

SUMMARY

Acetate oxidation by acetate adapted cells of Azotobacter was observed to be inhibited by ultraviolet light. This inhibition of a constitutive enzyme system was observed to be diminished by exposing irradiated cells to visible light. Adaptation of acetate grown cells to succinate was prevented by ultraviolet light. This irradiation effect was abolished by exposing irradiated cells to visible light. The significance of these observations is discussed.

REFERENCES


