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The Effect of Light on Xanthine Oxidase Activity

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It has been postulated, on the basis of studies of activation by light, that a flavin enzyme is involved in the indolylacetic acid oxidase system (Galston, 1950; Galston & Baker, 1951). The only report of light activation of a flavin enzyme appears to be that of Bernheim & Dixon (1928); working with a crude preparation of xanthine oxidase they found either activation or inactivation according to the conditions of the particular experiment. In a later study of the same enzyme only an inactivating effect of light was observed (Toyama, 1933). It has seemed important, therefore, to re-investigate the effect of light on xanthine oxidase.

EXPERIMENTAL

Enzyme. Milk from which casein had been removed after precipitation by rennin served as the source of enzyme in the majority of the experiments. In a few instances comparative tests were made with whole milk.

Substrate. Xanthine or hypoxanthine was employed as substrate. Preliminary studies indicated that maximal activity was obtained with 5×10^{-5} M-substrate.

Hydrogen acceptor. Methylene blue (MB), at 1×10^{-5} M, or nitrate, at 5×10^{-3} M, was used as hydrogen acceptor. The measurements were carried out in 0.05 M-phosphate buffer of pH 8.8 in the case of MB and of pH 5.5 with nitrate.

Measurement of enzyme activity. With MB, enzyme activity was measured by following spectrophotometrically with an Evelyn photoelectric colorimeter (620 m μ . filter) the time course of decolorization of the dye in evacuated Thunberg tubes. Activity was conveniently expressed as the reciprocal of the time required for 25 or 50 % reduction. To measure the rate of nitrate reduction, a series of identical

tubes was prepared and the nitrite determined successively in individual tubes, using a modification of the method of Parker (1949). The reaction rates were measured at 25° with MB and at 37° with nitrate.

Irradiation. The solution of enzyme in buffer, with or without hydrogen acceptor, was irradiated in open tubes immersed in a water bath at 25–27°, frosted tungsten filament lamps serving as the sources. The tubes were illuminated from two opposite sides and were shaken frequently. The non-irradiated control tubes, which were wrapped with aluminium foil, were manipulated concurrently in exactly the same way. At the highest intensities and longest exposures, the temperature of the illuminated solutions containing MB rose as much as 8° above that of the dark controls; tests showed that holding the enzyme solution in darkness at the elevated temperature did not affect its activity subsequently at 25°.

Procedure. Immediately after irradiation the tubes were closed and evacuated, the zero time transmission determined, the substrate tipped in, and readings commenced.

RESULTS

Light was found to influence the activity of xanthine oxidase only if an added photoreceptive pigment was present during the irradiation. Fig. 1 illustrates a typical experiment with MB.

Riboflavin also was found capable of serving as a photoreceptor in mediating light inactivation of xanthine oxidase. A comparison of the effectiveness of the two pigments was obtained as follows. Various amounts of riboflavin were added to portions of enzyme solution and MB to others. After irradiation at 1800 ft.c. for 5 min., MB was added to the riboflavin samples and the activities of all were measured in the usual manner. Riboflavin does not

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interfere with the spectrophotometric estimation of MB at 620 $m\mu$. In both cases the degree of inactivation was roughly proportional to the initial concentration of photoreceptor. For equal inactivation, about 2.5 times as great a concentration of riboflavin as of MB was required. It should be pointed out, however, that because of the much higher molecular extinction of MB, the total energy

radiation of about 500 ft.c. was without effect on the activity of the enzyme.

Figs. 2 and 3 present the results of experiments to ascertain whether activation might result from shorter exposure or lower intensity.

As these experiments gave no evidence of activation of the enzyme, in complete disagreement with those of Bernheim & Dixon (1928), a number of

Fig. 1. Influence of 30 min. irradiation at 1800 ft.c. on xanthine oxidase activity. Substrate, xanthine. Hydrogen acceptor, methylene blue. ○, non-irradiated; +, irradiated without methylene blue; ●, irradiated with methylene blue.

Fig. 2. Effect of light intensity on inactivation of xanthine oxidase. Exposure time, 30 min. Substrate, xanthine. Hydrogen acceptor, methylene blue.

absorbed was about 11.5 times as great as by the riboflavin. This factor was approximated by measuring the areas under the curves which were obtained by multiplying the molar absorption spectra, in the region 350–750 $m\mu$. (determined with a Beckman model DU spectrophotometer), by the emission spectrum of the source, as given by Taylor (1934).

By using nitrate as hydrogen acceptor, the dye may be eliminated entirely. In this case, exposure either to bright sunlight or to 'daylight' fluorescent

Fig. 3. Effect of duration of irradiation of xanthine oxidase. Intensity, 1800 ft.c. Substrate, xanthine. Hydrogen acceptor, methylene blue.

Fig. 4. Effect of added hydrogen peroxide on activity of xanthine oxidase. Substrate, xanthine. Hydrogen acceptor, methylene blue.

variations were tried in an attempt to discover the cause of the discrepancy. Inactivations entirely similar to the foregoing were obtained also with whole milk in place of the decaesinated preparation, with hypoxanthine rather than xanthine as substrate, at pH 6.8 and 7.4, and with various intervals of darkness interposed between the irradiation and measurement of activity.

Bernheim & Dixon (1928) believed that the effect of light was due to the formation of hydrogen

peroxide and presented data showing activation of xanthine oxidase on addition of small quantities of this substance in the dark. As shown in Fig. 4, we have been unable to reproduce this result either. It might be supposed that catalase, if present in the xanthine oxidase preparation, would tend to prevent activation either by hydrogen peroxide or by light, and indeed Bernheim & Dixon (1928) ascribed to the presence of catalase their failure to obtain activation with some of the milk samples tested. This explanation does not seem tenable, however, as we have obtained virtually identical inactivations, by light, of a xanthine oxidase preparation having high catalase activity and of one devoid of detectable catalase.

DISCUSSION

From the discrepancy between the foregoing results and those of Bernheim & Dixon (1928) it is evident that various crude preparations of xanthine oxidase may be affected entirely differently by illumination, whence it may be concluded that the influence of light is not directly upon the enzyme itself. As activations were never encountered in the present experiments, this indirect effect was not amenable to study, and no definitive explanation of the apparent activation observed by Bernheim & Dixon (1928) can be presented. However, one or two suggestions may be noted.

It is conceivable that light or hydrogen peroxide may diminish the effect of some inhibitor of the enzyme. In this connexion it is of interest that Kalckar & Klenow (1948) have shown that folic acid, which is known to occur in some samples of milk (Hodson, 1949), markedly inhibits the activity of xanthine oxidase. The inhibition was later ascribed to a decomposition product of folic acid and was shown to be relieved by irradiation (Lowry, Bessey & Crawford, 1949; Kalckar, Kjeldgaard & Klenow, 1950).

There exists also the possibility that the apparent

activation observed by Bernheim & Dixon (1928) was in reality quite unrelated to the activity of the xanthine oxidase, since any factor which accelerates reduction of the methylene blue or retards its re-oxidation would have the same result. The literature contains a number of reports of light activation of various dehydrogenases as measured by the Thunberg technique. That factors other than a true activation of the enzymes are involved is indicated by the observations that the extent of the accelerated decolorization is dependent upon the particular dye employed (Grönvall, 1937) and that accelerated reduction of MB may occur also with heat-inactivated enzyme preparations and in the absence of substrate (Krestownikoff, 1927). More recently, it has been shown that in the presence of MB, light brings about a dehydrogenation of certain amino-acids and other compounds with concurrent bleaching of the dye (Weil & Maher, 1950; Weil, Gordon & Buchert, 1951). It seems not unlikely that different samples of milk and of crude xanthine oxidase preparations might vary widely in the content of such photolabile substances.

Regardless of the precise mechanism which is involved, it is clear that the apparent activation of xanthine oxidase by light must be of an indirect nature, and hence cannot be adduced as evidence for the direct participation of a flavin enzyme in the indolylacetic acid oxidase system.

SUMMARY

1. Contrary to the results of Bernheim & Dixon (1928), no evidence could be obtained of an activation of xanthine oxidase by light.
2. It is suggested that the apparent activation observed by Bernheim & Dixon was of an indirect nature and may have been due to photodehydrogenation of impurities in their preparation, with concomitant reduction of the methylene blue employed as hydrogen acceptor.

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