PHOTOINACTIVATION OF TRANSKETOLASE OF BAKERS' YEAST

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Irradiation of transketolase by visible light in the presence of methylene blue leads to loss of its enzymic activity. The relationship between the rate of photoinactivation and the concentration of enzyme and of the sensitizer was investigated. The ionic strength has practically no effect on the photoactivation process.

Photooxidation is widely used in the study of the properties of enzymes. This method has been used by the writers to investigate functional groups of the transketolase of Bakers' yeast [1].

In the present investigation the effect of the experimental conditions on photoinactivation of the enzyme was studied.

EXPERIMENTAL METHOD

A crystalline specimen of transketolase was obtained from bakers' yeast by the method of Racker et al. [8]. Photooxidation was carried out in thermostatically controlled cells (22 ± 1°C) placed 21 cm from the source of radiation (a 200-W incandescent lamp, with a red filter). The thickness of the layer of the irradiated samples was 2 mm, their volume 0.5 ml, and pH 7.6. The composition of the samples (in moles) during irradiation (final concentrations) was as follows: glycyl-glycine buffer 0.1; transketolase $1.4 \cdot 10^{-6}$; methylene blue $2.5 \cdot 10^{-5}$. Similar tests were carried out, but without the photosensitizer, as the control. Activity was determined as described previously [1]. An enzyme sample with specific activity of 12 i.u. was used.

EXPERIMENTAL RESULTS

The rate of photoinactivation of transketolase as a function of methylene blue concentration is shown graphically in Fig. 1. With an increase in the concentration of photosensitizer (from $3 \cdot 10^{-7}$ to $0.6 \cdot 10^{-5}$ M) the rate of photoinactivation of the enzyme at first increased, then remained virtually unchanged over a wide range of concentration changes (from $0.6 \cdot 10^{-5}$ to $2.4 \cdot 10^{-5}$), after which it decreased gradually and fell to zero. The initial rise of the curve was due to the fact that the rate of photooxidation (other conditions being equal) is determined by the concentration of one of the substrates of the oxido-reduction reaction, in this case the concentration of methylene blue.* The subsequent fall of the curve on a further increase in the methylene blue concentration can evidently be explained by assuming that the sensitizer begins to limit the penetration of light into the interior of the solution, so that in fact the intensity of illumination

*Slowing of photooxidation in the presence of insufficient sensitizer has also been observed during the investigation of other enzymes [4, 6, 11].

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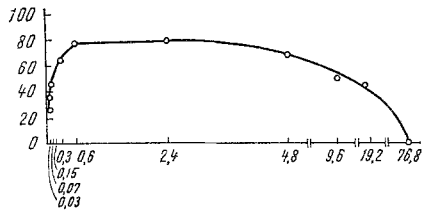

Fig. 1

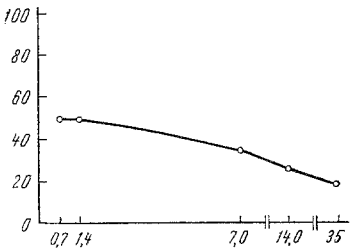

Fig. 2

Fig. 1. Photoinactivation of transketolase with different concentrations of methylene blue in a sample irradiated with light for 30 min. Abscissa – methylene blue concentration (in $M \cdot 10^{-5}$); ordinate – degree of photoinactivation (in % of initial activity).

Fig. 2. Photoinactivation of transketolase present in different concentrations in sample irradiated with light for 15 min. Abscissa – protein concentration (in $M \cdot 10^{-6}$); ordinate – degree of photoinactivation (in % of initial activity).

TABLE 1. Effect of Temperature on Velocity of Photoinactivation of Transketolase

Temperature, °C	pH	Degree of photoinactivation (in % of original activity)	Decrease in degree of photoinactivation at 2° compared with 22°C (photoinactivation at 22°C taken as 100%)
22	7,5	75	—
2	7,5	40	47
22	9,0	95	—
2	9,0	80	16

TABLE 2. Effect of Ionic Strength on Photoinactivation of Transketolase (duration of irradiation 15 min; methylene blue concentration $2.4 \cdot 10^{-5}$ M)

Salt added to sample	Concn. (in M)	Ionic strength	Degree of photoinactivation (in %)	Change in degree of photoinactivation (in %)
Nothing add.	—	—	55	—
KCl	2	2	42	—26
KNO_3	3,6	3,6	62	+12
NH_4NO_3	2,6	2,6	50	—9

falls and the rate of the process is slowed. Transketolase activity in the sample with methylene blue kept in darkness remained completely intact whatever the concentration of the sensitizer, indicating that the sensitizer itself had no inhibitory effect on the enzyme.

Relationship between Rate of Photoinactivation of the Enzyme and Its Concentration. The changes in the rate of photoinactivation of transketolase when present in different concentrations in the irradiated samples are illustrated in Fig. 2. The cause of the decrease in the rate of photoinactivation with an increase in the concentration of the enzyme is evidently the same as that during a decrease in the concentration of methylene blue: fewer molecules of the sensitizer are present for each molecule of protein. The possibility of mutual screening of the protein molecules, preventing the approach of excited molecules of the dye to them, likewise cannot be ruled out [3].

Photoinactivation of Transketolase at Different Temperatures. As the results in Table 1 show, the rate of photoinactivation fell with a decrease in temperature from 22 to 2°C, and more rapidly at pH 7.5 than at pH 9.0. The decrease in the degree of photoinactivation in the cold is explained by a decrease in the rate of photooxidation of the amino acid residues, destruction of which leads to loss of enzyme activity [5]. According to Weil [12], who investigated the temperature dependence of photooxidation of various amino acids, this dependence is most marked in the case of tyrosine. For instance, with a decrease in temperature from 20 to 2°C, the rate of tyrosine breakdown is reduced by almost five times. Meanwhile, the decrease in the rate of photooxidation of histidine was only 45-50%. This temperature dependence of photooxidation of amino acids evidently applies to enzymes also [13].

In the previous investigation [1] it was shown that photoinactivation of transketolase is due to destruction of the histidine residue (or residues) in the enzyme molecule. Meanwhile, the curve of velocity of photoinactivation versus pH indicated that the loss of activity at high pH values (8-10; the region of photooxidation of tyrosine residues) could be due to photooxidation not only of the histidine, but also of the tyrosine residues. However, since with a change in temperature from 22 to 2°C the observed decrease in the rate of photoinactivation at pH 7.5 was higher than at pH 9.0 (Table 1), this explanation of the increase in the rate of photoinactivation of the enzyme in the alkaline region [1] by photooxidation of tyrosine residues seems doubtful.

Effect of Ionic Strength. The work of Bellin and Jankus [2] has shown that ionic strength has no effect on the photooxidation of free amino acids. In the case of protein, however, ionic strength may influence its structure and thus change the reactivity of the photooxidized amino acid residues. Interaction of the ions directly with the photooxidized groups of the protein or with the molecule of the sensitizer is also a possibility. For instance, the presence of paramagnetic cations or halogens during photoirradiation of taka-amylase inhibited its photoinactivation, by shortening the period during which the sensitizer (riboflavin) was in an excited state [7, 9, 10]. The results of the investigation of the effect of ionic strength on the photoinactivation of transketolase are given in Table 2. Of all the salts investigated only KCl had any appreciable effect on the rate of photoinactivation of the enzyme. However, methylene blue was strongly decolorized in the presence of KCl (both in the experimental sample and in the control – in darkness): according to the photometric data, by 10 times during an experiment lasting 15 min. Special experiments to study photoirradiation of transketolase in the presence of sensitizer in a concentration of $2.4 \cdot 10^{-6}$ M demonstrated a decrease in the rate of photoinactivation of 27% in 15 min, i.e., about the same as in the experiments with KCl described above, when methylene blue was used in a concentration of $2.4 \cdot 10^{-5}$ M (Table 2). It can thus be concluded that a high ionic strength had virtually no effect on the rate of photoinactivation of transketolase.

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