INHIBITORY EFFECT OF SALICYLALDOXIME ON CHLOROPLAST PHOTOOXIDATION-REDUCTION REACTIONS*

by

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The inhibitory effect on photosynthesis of reagents including salicylaldoxime which are believed to react specifically with copper enzymes was first described by Green et al (1939). Whereas both photosynthesis and respiration of Chlorella pyrenoidosa were inhibited, photosynthesis was more sensitive to the inhibitors.

Trebst (1963) found that 10^{-2} M salicylaldoxime inhibited the Hill reaction of spinach chloroplasts with ferricyanide or NADP as oxidant, as well as photophosphorylation with menadione as cofactor, but not the photoreduction of NADP with ascorbate and DPIP as electron donor. He assumed that salicylaldoxime interfered with electron transfer between the oxygen evolving system and cytochrome f, probably at the site of plastocyanin.

Fork and Urbach (1965) investigated the effect of salicylaldoxime

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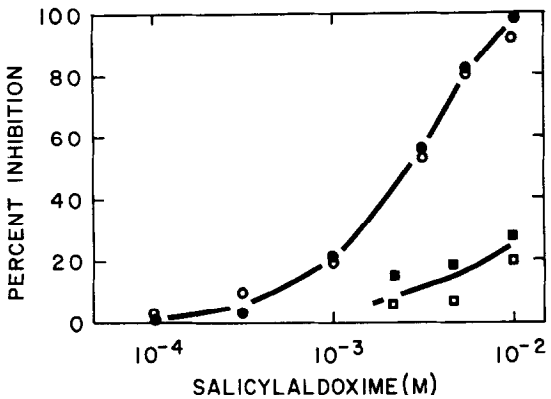
on several light-induced absorbance changes in Ulva lobata. The poison eliminated the absorbance change at 591 mμ which they assumed to be associated with plastocyanin, but not the absorbance change at 419 mμ which was indicative of cytochrome f photooxidation. The reduction of cytochrome f which occurred during the red light illumination was markedly suppressed by the poison. They proposed an electron transfer chain wherein plastocyanin was located between cytochrome f and the oxygen evolving system.

The results described herein relate to the inhibition by salicylaldoxime of photooxidation-reduction reactions with untreated and sonically treated chloroplasts. The reaction of the copper in plastocyanin with salicylaldoxime was also studied.

The preparation of spinach chloroplasts and sonic treatment of the chloroplasts have been described (Katoh and Takamiya, 1965; Katoh and San Pietro, in press). Plastocyanin was prepared as described by Katoh et al (1963). The Hill reaction with 2,6-dichlorophenol indophenol (DPIP) as oxidant and the photoreduction of methyl red with ascorbate and DPIP as electron donor were measured spectrophotometrically (Katoh and Takamiya, 1965).

The effect of salicylaldoxime on the Hill reaction of chloroplasts with DPIP is shown in Fig. 1. A high concentration of the poison was necessary to inhibit significantly the Hill reaction; almost complete inhibition was attained with 10^{-2} M salicylaldoxime.

When chloroplasts are subjected to sonic treatment, the interaction between photosystems 1 and 2 (terminology of Duysens [1962]) is interrupted and the plastocyanin is solubilized. In spite of these effects, sonication does not alter drastically the Hill activity with DPIP; that is, the photoreduction of the dye appears to require only photosystem 2. The Hill activity of sonicated chloroplasts was more sensitive to salicylaldoxime than that of untreated chloroplasts when



LEGEND OF FIGURE 1 Effect of salicylaldoxime on the Hill reaction and photoreduction of methyl red with ascorbate and DPIP with untreated and sonically treated chloroplasts.

Each reaction mixture contained, in a final volume of 3.0 ml, 150 μmoles of phosphate, pH 7.0, 30 μmoles of NaCl, chloroplasts, or chloroplasts treated sonically for 10 minutes, equivalent to 30 μg of chlorophyll, and 0.1 μmole of DPIP. Hill reactions were carried out in a cuvette (1 x 1 x 4 cm) under white light of 3000 foot candles. Photoreduction of methyl red was carried out anaerobically using a Thunberg type cuvette under red light of saturating intensity. Each reaction mixture was the same as that for the Hill reaction and, in addition, contained 3 mμmoles of 3-(3,4-dichlorophenyl)-1,1-dimethylurea, 20 μmoles of neutralized ascorbic acid, 0.2 μmole of DPIP and 0.08 μmole of methyl red. Plastocyanin (5 mμmoles) was added to the treated chloroplasts. Bleaching of the dyes caused by illumination was determined spectrophotometrically. Hill reaction with chloroplasts (O) or sonically treated chloroplasts (●). Methyl red photoreduction with chloroplasts (Δ) or with sonically treated chloroplasts (▲).

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the measurements were made at pH's where the activities of the two preparations were optimal; that is, pH 5.5 and 7.5 for sonicated and untreated chloroplasts, respectively. This apparent difference

in sensitivity was, however, due to a pH-dependency of the inhibition and not related to the respective chloroplast preparations; the more acidic was the pH of the reaction mixture, the more inhibitory was the poison. In fact, as shown in Fig. 1, there was no difference in sensitivity of the Hill reaction of untreated and sonicated chloroplasts to salicylaldoxime when they were assayed at the same pH (pH 7.0). It is concluded, therefore, that salicylaldoxime inhibits photosystem 2 or some reaction which is closely related to photosystem 2.

Fork and Urbach (1965) observed an appreciable suppression of the light-induced absorbance change in Ulva only after preincubation of the algal thalli with salicylaldoxime. However, inhibition of the Hill reaction occurs almost instantaneously both with the untreated and the sonically treated chloroplasts. The extent of inhibition was unaltered by preincubation of the chloroplasts with the poison at room temperature for a period up to 25 minutes, provided that the measured activities were corrected for the gradual inactivation of control chloroplasts which were incubated similarly but without the poison.

It has been shown previously (Katoh and Takamiya, 1965; Katoh and San Pietro, in press) that the sonic treated chloroplasts required plastocyanin for the ascorbate-DPIP supported photoreduction of NADP or methyl red. It was of special interest to study the effect of salicylaldoxime on this plastocyanin-dependent photoreduction. Methyl red was used as the electron acceptor because of the difficulty in measuring NADP photoreduction in the presence of high concentration of the poison which has a high absorbance around 340 mμ. The photoreduction of methyl red with the untreated chloroplasts in the absence of added plastocyanin, as well as the sonically treated chloroplasts in the presence of plastocyanin, was much less sensitive to salicylaldoxime than the Hill reaction (Fig. 1). This

is in agreement with the finding of Trebst (1963) that electron flow from reduced DPIP to NADP was not inhibited by salicylaldoxime. These results show that salicylaldoxime cannot be used as a specific inhibitor for plastocyanin since the photoreduction of NADP by ascorbate and DPIP requires plastocyanin and is insensitive to salicylaldoxime.

This conclusion is supported by the results of experiments relating to the reactivity of the copper of plastocyanin with salicylaldoxime. Removal of copper from plastocyanin by dialysing the protein overnight against a high concentration of salicylaldoxime has been reported (Katoh, 1960). In the present work, a shorter time of reaction was used. Oxidized and reduced plastocyanin were incubated with 10^{-2} M salicylaldoxime in 0.05 M phosphate, pH 7.0, at room temperature. The absorption spectrum of the protein, oxidized, if necessary, with potassium ferricyanide was determined after an appropriate time of incubation.

Treatment of reduced plastocyanin with salicylaldoxime for 30 minutes caused no appreciable change in the absorption spectrum of the protein. Prolonged incubation (24 hours), however, resulted in a decrease in absorbance at 597 mμ to approximately half of the original value. When oxidized plastocyanin was treated for one hour complete bleaching of its blue color occurred. The addition of ferricyanide to the bleached protein restored most of the original absorption of the protein, thereby indicating that the rapid bleaching observed was due mainly to the reduction of the copper. In the presence of an amount of ferricyanide sufficient to keep the protein in the oxidized form, a gradual and irreversible decolorization of the protein was observed. This decolorization amounted to about 50 percent after 2 hours and 100 percent after 24 hours. This bleaching reflects only the decrease in the magnitude of the absorbance without any significant change in the shape of the absorption spectrum. It is

clear, therefore, that the reaction of the copper in plastocyanin with salicylaldoxime is too slow to account for the inhibiting action of the poison on photosynthetic reactions in term of a specific interaction with plastocyanin.

The reactivity of copper in plastocyanin with several other chelating reagents was studied similarly. These included sodium diethyldithiocarbamate, potassium ethyl xanthate, thiourea, 2,2'-biquinolyl, neocuproine, o-phenanthroline, 8-hydroxyquinoline and potassium cyanide. None of them reacted with the copper of plastocyanin rapidly nor completely as to be useful as an inhibitor of the copper protein.

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