EFFECT OF SALICYLALDOXIME ON THE COMPLETE ELECTRON TRANSPORT SYSTEM OF PHOTOSYNTHESIS AND ON THE ISOLATED REACTION CYCLE II

by

G. Renger, J. Vater and H. T. Witt

Max-Volmer-Institut, I. Institut für Physikalische Chemie der Technischen Universität Berlin

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Green et al. (1939) observed that copper-complexing agents like salicylaldoxime are inhibitors for photosynthesis and respiration in chlorella.

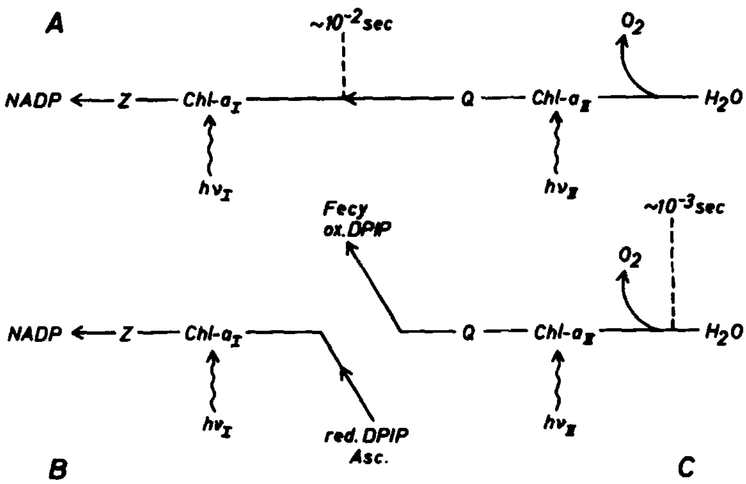

Fig. 1 A Complete electron transport system of photosynthesis (cycle I + II) (H_2O as electron donor and NADP as electron acceptor)
B Isolated reaction cycle I (red. DPIP + ascorbate as electron donor)
C Isolated reaction cycle II (ox. DPIP + $K_3[Fe(CN)_6]$ as electron acceptor)
NADP = Nicotinamide adenine dinucleotide phosphate; Z = Experimentally detected but chemically unknown intermediate; Chl-a_I = Chlorophyll-a_I ; Q = Plastoquinone ; Chl-a_{II} = Chlorophyll-a_{II} ; DPIP = 2, 6-Dichlorophenolindophenol ; Asc = Ascorbate ; Fecy = $K_3[Fe(CN)_6]$.

Trebst (1963) found that salicylaldoxime inhibits the complete electron transport system of photosynthesis (reaction cycle I + II) with NADP as electron acceptor and H_2O as electron donor (fig. 1 A), but not the isolated reaction cycle I with DPIP and ascorbate as electron donor (fig. 1 B).

Fork et al.(1965) deduced from the quenching of the light-induced absorbance change at 591 mμ with salicylaldoxime that this poison blocks specifically the reactivity of plastocyanin. Plastocyanin contains two atoms of copper and has been isolated by Katoh (1960). It is suggested that plastocyanin is located between cycles I and II.

In this paper the action of salicylaldoxime on a) the complete electron transport system (reaction cycles I + II) and b) on the isolated reaction cycle II is investigated. If salicyclaldoxime blocks specifically a plastocyanin reaction between the cycle I and II, it is expected that the complete system is inhibited by salicylaldoxime, but not the isolated cycle II (fig. 1c).

By addition of oxidized DPIP and $K_3[Fe(CN)_6]$ it is possible to isolate the cycle II chemically from the complete reaction system (cycle I + II) (Rumberg et al. 1963). This can be achieved by blocking the cyclic electron flow around Chl-a_I through interception of electrons from Z^- with $K_3[Fe(CN)_6]$ and by decoupling of the linear electron flow from H_2O to Chl-a_I through interception of electrons from Q^- with oxidized DPIP. The degree of isolation of cycle II depends α) on the concentration of $K_3[Fe(CN)_6]$ and DPIP, but also strongly β) on the intensity γ) duration and δ) frequency of the illumination (Witt, Skerra et al. 1965, Rumberg et al. 1965).

A criterion for a complete isolation of cycle II is firstly the disappearance of the absorption change of Chl-a_I in cycle I at 703 mμ and secondly the change from the rate determining step of the complete system (cycle I + II) which is 10^{-2} sec to the rate determining step of the isolated cycle II which is only 10^{-3} sec. (Witt, Rumberg et al. 1965)

To obtain unambiguous results on the one hand the measurements were carried out with spinach chloroplasts in presence of salicylaldoxime. On the other hand chloroplasts were incubated in the salicylaldoxime containing solution, afterwards centrifugated and resuspended in fresh reaction medium without salicylaldoxime. The oxygen production was measured manometrically. The amount of oxygen per flash in dependence of the ratio (molecules of salicylaldoxime) / (molecules of chlorophyll) is shown in fig. 2.

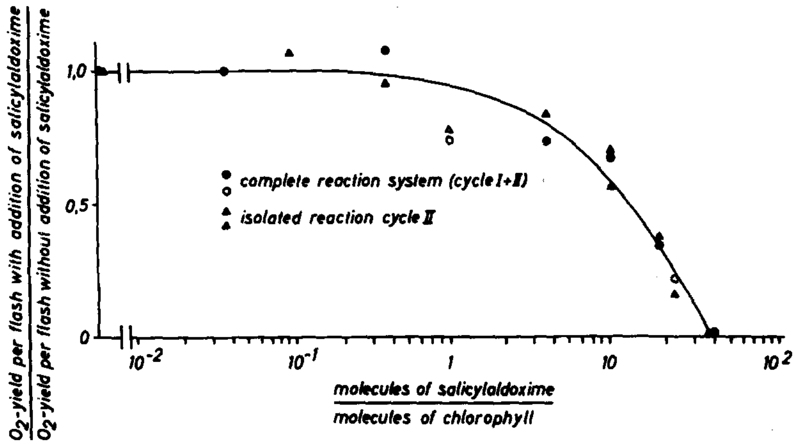

Fig. 2 Relative oxygen yield per flash on spinach chloroplasts as a function of the ratio (molecules of salicylaldoxime) / (molecules of chlorophyll).
Open and solid circles: Complete reaction system (reaction cycle I + II).
$2,8 \cdot 10^{-4}$ M/l chlorophyll; $5,5 \cdot 10^{-3}$ M/l K_3 $[Fe(CN)_6]$; 0,05 M/l Tris-HCl,
Open and solid triangles: Isolated reaction cycle II. $2,8 \cdot 10^{-4}$ M/l chlorophyll; $5,5 \cdot 10^{-3}$ M/l K_3 $[Fe(CN)_6]$; 10^{-4} M/l ox. DPIP ; 0,05 M/l Tris-HCl, pH = 7,2.
Solid circles and triangles: Chloroplasts in presence of salicylaldoxime.
Open circles and triangles: Chloroplasts after separation from salicylaldoxime. The chloroplasts were incubated for 10 minutes in the salicylaldoxime containing solution and after centrifugation resuspended in fresh reaction solution without salicylaldoxime.
Illumination: Periodic flashes. Flash duration: $5 \cdot 10^{-5}$ sec. Saturating light intensity. Dark time between the flashes: 10^{-1} sec. Time of illumination: 7,5 min (4500 flashes).
Temperature: 20 °C.
O_2-yield per flash: $2,1 - 2,7 \cdot 10^{-10}$ M/mg Chl (unpoisoned chloroplast preparations)

According to fig. 2 the action of salicylaldoxime on the oxygen production in the complete reaction system (cycle I + II) is the same as in the isolated cycle II. It is concluded, therefore, that either salicylaldoxime inhibits a plastocyanin independent reaction step in the isolated reaction system II or that plastocyanin is localized in the isolated cycle II. The last possibility is, however, unlikely, because in the isolated cycle II no absorbance changes are found which could be caused by plastocyanin. Consequently salicylaldoxime is not a specific inhibitor for plastocyanin. Therefore all results concerning the function of plastocyanin in connection with salicylaldoxime are doubtful. These results are in agreement with those of recent experiments of Katoh and San Pietro (1966) on intact and sonicated chloroplasts.

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