# Evidence for a Rapid Oxygen-Uptake in Tobacco Chloroplasts

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A fast oxygen uptake, induced by a sequence of short (5 μsec) saturating flashes was observed in chloroplasts of wild type tobacco and two chlorophyll-deficient tobacco mutants. One of the chlorophyll mutants is the earlier described variegated tobacco NC 95. Chloroplasts of this mutant exhibit only photosystem I mediated photoreactions, hence the observed oxygen uptake is to be associated with photosystem I. This is further substantiated by the fact that the oxygen uptake is insensitive to DCMU in the two chloroplast types used, which have both photosystems fully functioning. The uptake depends on the addition of electron acceptors like *p*-benzoquinone in intact chloroplasts or on *p*-benzoquinone or ferricyanide in chloroplasts that have lost the envelope. In dark adapted chloroplasts, therefore, under these conditions the overall apparent gas exchange in the first two flashes is consumption. Although the uptake is slower than photosynthetic oxygen evolution it clearly affects the oxygen yield in the flash sequences. This is demonstrated by several experiments in which the apparent oxygen consumption in the absence of DCMU oscillates with a periodicity of four. We have indications that in chloroplasts of the tobacco aurea mutant *Su/su* the oxygen uptake is faster than in wild type chloroplasts.

Photorespiration is a wasteful process which counteracts high rates of apparent photosynthesis and therefore reduces many crop yields substantially. The overall process is certainly a mixture of several phenomena but manifests itself as simple as by an evolution of $CO_2$ and an uptake of $O_2$ in the light during photosynthesis. Ultimately, not much is understood of this process. Somehow glycolate, an early product of photosynthesis, and its decomposition by the enzyme glycolate oxidase play a role, or $O_2$-uptake, mediated by the enzyme ribulose 1,5-biphosphate oxygenase, seems to account for some of the light dependent reversal of photosynthetic gas exchange [1, 2].

In the attempt to understand and to control photorespiration, we have genetically and functionally characterized several tobacco mutants [3, 4], which differ with respect to their photorespiratory activity and their photosynthetic unit size.

In the following we report on a rapid $O_2$-uptake in tobacco chloroplasts which under certain conditions might affect the yield of photosynthetic oxygen evolution.

*Abbreviations:* DCMU, N-N′-3,4-dichlorophenyl dimethyl aurea.

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## Materials and Methods

*Chloroplasts* of the different tobacco strains were prepared according to Homann and Schmid [5]. The chloroplasts are osmotically swellable and are for a short time after the preparation intact [5]. The chloroplasts were suspended in a buffer containing 0.4 M sucrose, 0.05 M Tris pH 7 and 0.01 M NaCl, 0.2% serum albumin (Merck, Germany) and 0.2% pectinase (Sigma, USA).

*Oxygen Measurements.* The measurements of oxygen uptake and evolution were carried out by polarography with three electrodes connected in the set-up shown in Figs 1 a and b. 1 ml of the chloroplast suspensions is directly put on the two electrodes Pt and Ag/AgCl. The platinum electrode has in comparison to the reference electrode Ag/AgCl a potential of −0.6 V. The potential is kept constant with a third silver electrode which allows for the necessary electric current in the circuit. The regulation is done by means of an operational amplifier LH 0044 H of National Semiconductor (Fig. 1 a). The contact between the reference and the silver electrode is assured by means of a 1 N KCl bridge which is put to operation by means of a syringe applied at site 5 of the scheme of Fig. 1 b. This type of apparatus has been designed in order to work also with leaf sections which are directly put in contact with the electrode.

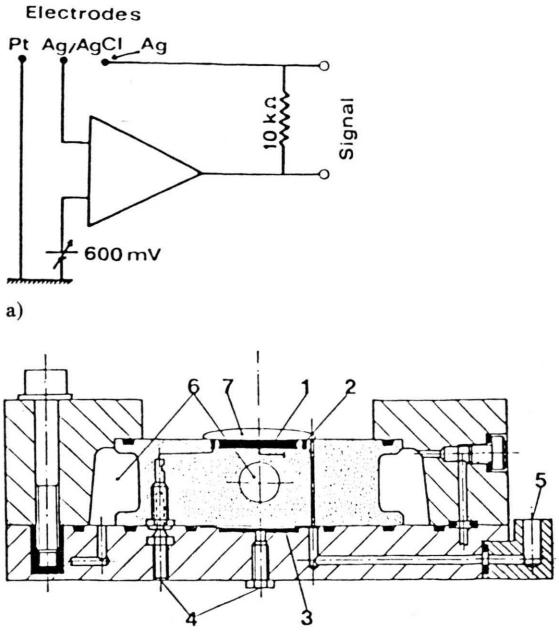


Fig. 1. a) Electronic circuit scheme of the oxygen electrode used. b) Schematic diagram of the set-up: 1) Platinum electrode; 2) reference electrode Ag/AgCl; 3) auxiliary silver electrode; 4) electric connections; 5) site of application of potassium chloride; 6) jacket for the thermoregulation; 7) chloroplast suspension.

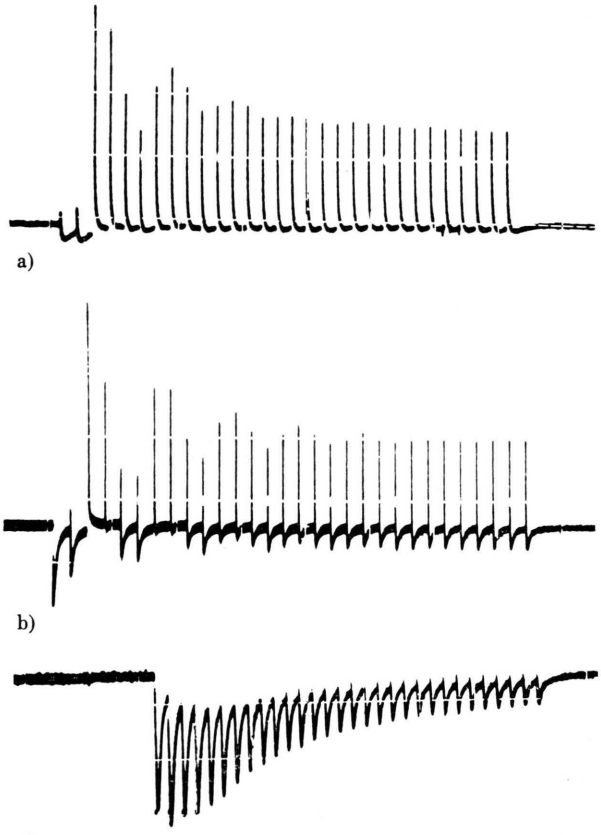


Fig. 2. Oxygen yield pattern for a series of 30 flashes in dark adapted (15—30 min) chloroplasts of wild type tobacco *N. tabacum* var. John William's Broadleaf. a) Without additions; b) in the presence of $10^{-3}$ M *p*-benzoquinone, note the oscillating oxygen uptake below the base line; c) in the presence of $10^{-3}$ M *p*-benzoquinone plus $10^{-6}$ M DCMU.

*Flashes* were provided by a Stroboscope 1545 of General Radio. The flash duration was 3 $\mu$ sec at the half intensity height. Usually, a sequence of 30 flashes spaced 300 msec apart was given.

## Results

### *Oxygen exchange in chloroplasts of the wild type tobacco*

Dark adapted tobacco chloroplasts show when illuminated with a sequence of short saturating flashes the oscillating $O_2$-evolution (Fig. 2 a) well known from the literature [6, 8]. Careful examination of this evolution pattern reveals that under the first two flashes besides a slight oxygen evolution also a fast oxygen uptake is distinctly visible (Fig. 2 a). However, in the presence of ferricyanide or better $10^{-3}$ M *p*-benzoquinone the first two flashes induce a substantial stimulation of the fast apparent $O_2$-uptake (Fig. 2 b). From there onward *i. e.* the 3rd, 4th flash etc. the oscillating $O_2$-evolution is preponderant. In further experiments it became obvious that in the apparent $O_2$-evolution an uptake is contained a fact which is best demonstrated in the assay in the presence of *p*-benzoquinone and DCMU (Fig. 2 c) and by using chloroplasts from the tobacco aurea mutant Su/su characterized in earlier reports [4, 5, 9, 10].

### *Oxygen uptake in chloroplasts of the tobacco aurea mutant Su/su*

The mutant is characterized by a reduced photosynthetic unit size [5, 9, 11] and a high photorespiratory activity [4, 10, 12]. Chloroplasts prepared from mutant leaves show, when dark adapted in the experimental conditions of Fig. 2 a, essentially the same oscillating $O_2$-evolution as the wild type. In the presence of *p*-benzoquinone, however, the $O_2$-uptake under the first two flashes is drastically increased when compared in relation to the sub-

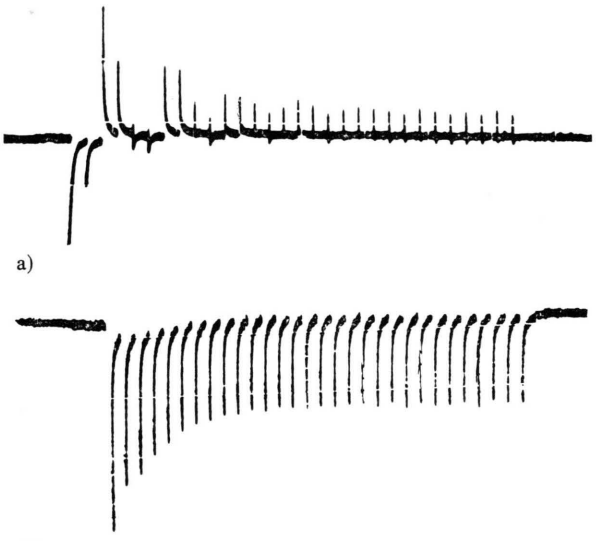


Fig. 3. Oxygen exchange pattern for a series of 30 flashes in dark adapted chloroplasts of the tobacco aurea mutant *N. tabacum* var. *Su/su*. a) In the presence of $10^{-3}$ M *p*-benzoquinone; b) in the presence of $10^{-3}$ *p*-benzoquinone plus $10^{-6}$ M DCMU.

sequent apparent $O_2$-evolution in the 3rd and 4th flash (Fig. 3 a). As is seen from Fig. 3 a, from the third flash onward the gas exchange is outbalanced by the $O_2$-evolution but the $O_2$-uptake which is nevertheless contained in the $O_2$-evolution sequence clearly appears upon addition of DCMU (Fig. 3 b). From this data it follows that the $O_2$-uptake has to be associated with light reaction I and *p*-benzoquinone apparently serves as an electron acceptor in the reaction.

As in earlier studies we had shown that chloroplasts of yellow leaf sections of the variegated tobacco NC 95 [5] exhibited only photoreactions assocaited with photosystem I we felt that these chloroplasts could provide further insights.

*Oxygen uptake in chloroplasts of yellow leaf sections of the variegated tobacco NC 95*

Chloroplasts of this tobacco mutant exhibit only photosystem I mediated photoreactions such as the photoreduction of $NADP^+$ or of an artificial acceptor like anthraquinone-2-sulfonate with dichlorophenol indophenol/ascorbate as the electron donor [5, 13]. Cytochrome *f* and plastocyanin seem to be functioning in these chloroplasts [14, 15] but there is no $O_2$-evolution [5] or primary photochemistry of photosystem II (without $O_2$-evolution) as shown *e. g.* by low temperature fluorescence [16]. The flash treatment of such chloroplasts causes no $O_2$-evolution as is expected from our earlier data [5], but rather an overall slight oxygen-uptake (Fig. 4 a) which is totally insensitive to DCMU (Fig. 4 b). The presence of $10^{-3}$ M *p*-benzoquinone, however, induces an appreciable uptake (Fig. 4 c) which again is insensitive to DCMU (Fig. 4 d).

The $O_2$-uptake in the presence of $10^{-3}$ M *p*-benzoquinone depends on the spacing of the flashes. Spacing the flashes 2 sec apart shows that more than 90 per cent of the electron donating pool have

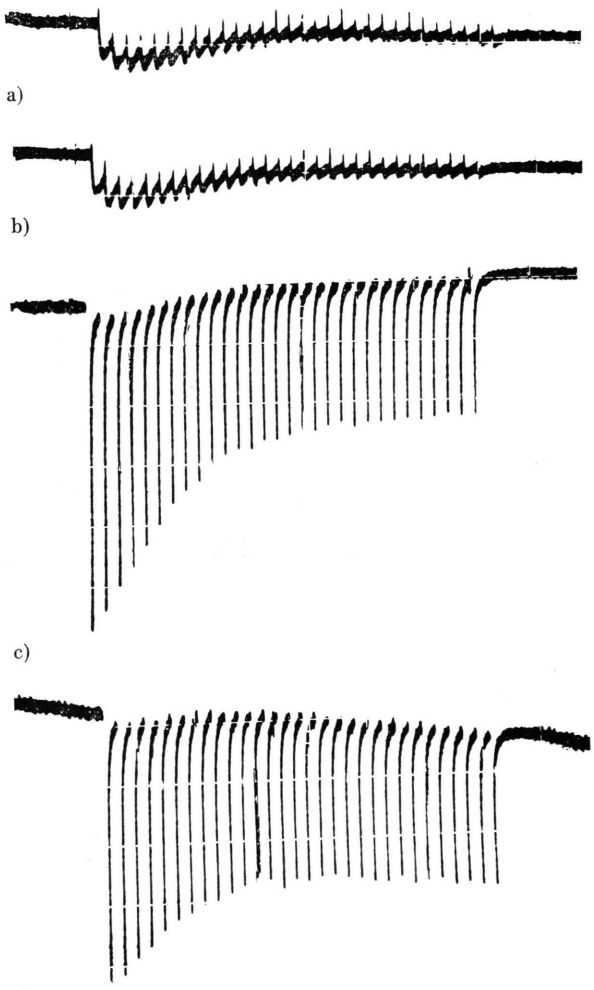


Fig. 4. Oxygen uptake pattern for a series of 30 flashes in dark adapted chloroplasts of yellow leaf sections of the variegated tobacco mutant *N. tabacum* var. NC 95. a) No additions; b) plus $10^{-6}$ M DCMU; c) in the presence of $10^{-3}$ M *p*-benzoquinone; d) in the presence of $10^{-3}$ M *p*-benzoquinone plus $10^{-6}$ M DCMU.

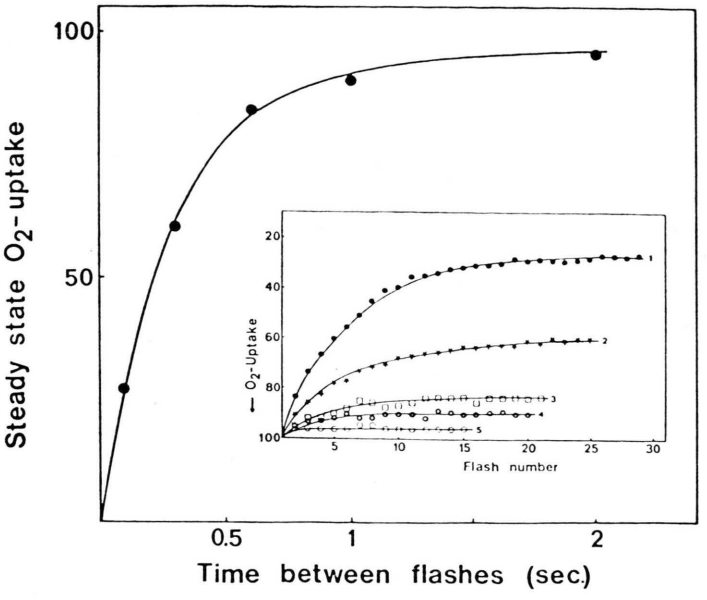


Fig. 5. Steady state of the yield of oxygen uptake in per cent of the maximal yield produced by the first flash in dependence on the dark time between flashes. The experiments were carried out in the presence of $10^{-3}$ M *p*-benzoquinone. The plot is for chloroplasts of the variegated tobacco mutant NC 95 and was obtained for a series of usually 30 flashes for each shown intervall. The inset shows the oxygen uptake profile for the individual dark times between the flashes. 1) 100 msec; 2) 300 msec; 3) 600 msec; 4) 1 sec; 5) 2 sec.

recovered or are filled up. The half life time for the reaction which uses up the reducing power of photosystem I and which manifests itself as $O_2$-uptake is around 200 msec (Fig. 5).

## Discussion

The discussion in this paper is restricted to the question of how this light induced $O_2$-uptake is produced. It appears that normal chloroplasts without additions show an endogenous $O_2$-uptake in a very attenuate form even though the uptake is clearly present. The large effect reported on in this paper is caused by the addition of compounds such as *p*-benzoquinone and ferricyanide, which in principal can only react as electron acceptors. One could visualize that the photoreduction of *p*-benzoquinone directly leads to an autooxidisable product which then would account for the $O_2$-uptake. However, this explanation is certainly not sufficient because ferricyanide essentially causes the same effect (data not shown) which implies that electrons from photoreduced ferricyanide (*i.e.* ferrocyanide) must be transferred to an endogenous electron acceptor in the chloroplast which in its reduced form is autooxidisable and then causes the $O_2$-consumption in a Mehler type reaction. The same must be true for *p*-benzoquinone. Hence, both compounds ferricyanide and *p*-benzoquinone serve under the presented experimental conditions as electron acceptors on the acceptor side of photosystem I which, according to the literature and from the concentrations used is not self evident but which appears justified due to our observations with chloroplasts of NC 95 (Fig. 4), which have no photosystem II activity. Concerning the effect of ferricyanide, it should be mentioned that in contrast to *p*-benzoquinone the compound serves only as an acceptor in chloroplasts which have lost their envelope which means that in fully intact chloroplasts ferricyanide does not penetrate into the organel and therefore does not induce an $O_2$-uptake.

The authors feel that the light induced oxygen uptake reported on in this paper might be one of the major means of chloroplasts to dispose of their excess reducing power *in vivo*.

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