# VECTORIAL ELECTRON FLOW ACROSS THE THYLAKOID MEMBRANE. FURTHER EVIDENCE BY KINETIC MEASUREMENTS WITH AN ELECTROCHROMIC AND ELECTRICAL METHOD

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Received 30 October 1973
Original figures received 3 December 1973

## 1. Introduction

In photosynthesis the electron flow from $H_2O$ to $NADP^+$ occurs by two light reactions at the centers chlorophyll-$a_I$-700 [1] and chlorophyll-$a_{II}$-680 [2]. A pool of plastoquinone PQ is the link between the light reaction centers [3]. The electron transfer is accompanied by the formation of an electrical potential difference $\Delta\varphi$ [4]. $\Delta\varphi$ Has been measured by field indicating absorption changes, i.e. by the shift of the absorption bands of the membrane pigments in a field (electrochromism) [4–6]. From this potential difference it has been concluded that the electron transfer is a vectorial transfer from the inside of the thylakoid membrane to the outside [4] (fig. 1). The formation of the potential difference occurs together with the photoact in $\tau(\Delta\varphi) < 20$ nsec [7]. At each light reaction one half of the potential is set up: $\Delta\varphi_I = \Delta\varphi_{II}$; $\Delta\varphi_I + \Delta\varphi_{II} = \Delta\varphi$ [8]. The charges on the outside ⊟ and inside ⊞ are replaced by $OH^-$ and $H^+$ through redox reactions. This corresponds to one $H^+$ translocation into the inner space of the thylakoid at each light reaction [8]. The decay of $\Delta\varphi$ by $H^+$ efflux is coupled with the formation of ATP [4, 9–11]. For review see [12]. In respect to phosphorylation these results are in agreement with a hypothesis of Mitchell [13].

One essential event in the described reaction sequence is the vectorial electron flow. This communication deals with two measurements which confirm in an independent way the orientated flow particularly at Chl-$a_{II}$. Basis for such measurements are the following properties. The electrical potential difference $\Delta\varphi_{II}$ induced by light reaction II increases proportionally to the number of electrons injected into the pool of PQ [14, 15]. The proton uptake induced by light reaction II increases also proportionally to the number of electrons which are injected into the pool of PQ [15]. These results substantiate the proposal of a transmembrane electron shift from $H_2O$ (inside) to PQ (outside) and an $H^+$ uptake at PW (outside) and $H^+$ release from $H_2O$ (inside) as depicted in the zigzag scheme of fig. 1. The transfer time for one electron from $H_2O$ to the PQ pool is 0.6 msect at 20°C. This value has been estimated from the time which is necessary for a recycling of $H_2O$ oxidation, i.e. $O_2$ production [16] and PQ reduction [3]. This time is the same as it has been measured for the half life time $\tau(X{-}320)$ of a substance $X{-}320$ with absorption changes at 320 nm [3, 17]:

$$\tau(0_2) = \tau(X{-}320) = 0.6 \text{ msec}$$

$X{-}320$ is probably a special plastosemiquinone $PQ^-$ complexed with Chl-$a_{II}$ [3] and in the oxidized form the primary electron acceptor of Chl-$a_{II}$ because $X{-}320$ can be trapped at cryogenic temperature [18]. These results indicate that the release of the electon from $X{-}320$ to the PQ pool within 0.6 msec is the overall transfer time of the electron e from $H_2O$ to the PQ pool [3]:

$$\tau(H_2O \xrightarrow{e} PQ) = 0.6 \text{ msec}$$

When this electron transfer occurs vectorially across the membrane, which means generation of the electrical potential $\Delta\varphi_{II}$, the transfer time 0.6 msec should be identical with the time necessary to pass away before

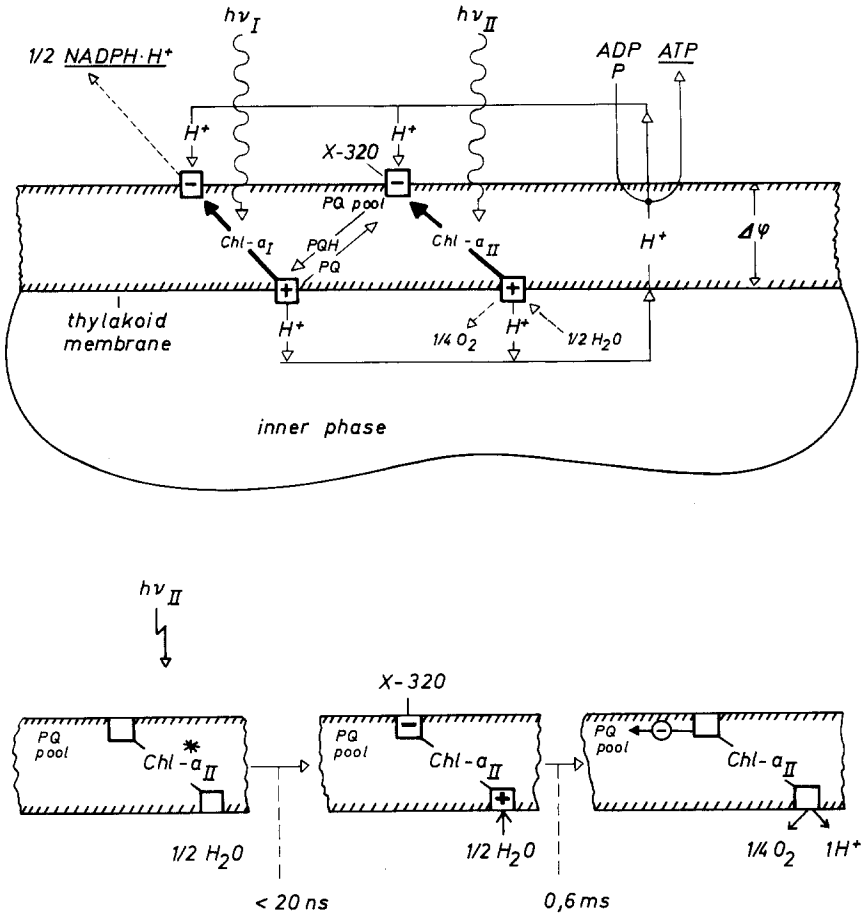


Fig. 1. *Top*: Vectorial electron transfer and proton translocation in a thylakoid membrane during photosynthesis. *Bottom*: Reaction sequence of the vectorial electron transfer particulary at light reation II. For details see text.

$\Delta\varphi_{II}$ can be generated a second time. In the following we have measured this recovery time of $\Delta\varphi_{II}$. The observed identity of this time with the transfer time 0.6 msec supports the assumption of a vectorial electron flow across the membrane.

## 2. Methods

The recovery time for $\Delta\varphi_{II}$ has been estimated as follows. The flash induced extent of the electrical potential difference $\Delta\varphi_{II}$ induced by Chl-$a_{II}$ was measured as a function of the dark time $t_d$ between a double flash. The time in which $\Delta\varphi_{II}$ decreases to 50% was taken as the characteristic recovery time $\tau$. To be able to measure $\Delta\varphi_{II}$ alone, the formation of $\Delta\varphi_I$ induced by Chl-$a_I$ has been eliminated with 720 nm continuous background light. This light excites predominantly Chl-$a_I$. At appropriate intensity light reaction I is saturated and the signal induced by the flash is practically only due to light reaction II [21]. The potential difference $\Delta\varphi_{II}$ in a double flash has been measured in two ways: (i) *Optically* by the field indicating absorption changes (see above) and (ii) *electrically* by macroscopic electrodes (details see below). The *optical* measurement by electrochromism – $\Delta\varphi_{II}^{opt}$ – has been carried out at the wavelength at which the electrochromic signal is maximal, that is at 515 nm. The *electrical* measurement – $\Delta\varphi_{II}^{el}$ – is based on the effect that in a *non* saturating flash, fired e.g., from the top of the cuvette, the thy-

lakoids are asymmetrically charged. This is because in the upper part of each thylakoid more light is absorbed than in the lower part. The difference of $\Delta\varphi_{II}$ between the upper and lower part is proportional to $\Delta\varphi_{II}$ and induces an electrical signal at 2 macroscopic electrodes which are located near the top and the bottom of the cuvette (distance e.g. $d \approx 1$ cm) [19, 20]. The first flash of the double flash has been chosen of saturating intensity. This flash charges each place at each light reaction. The second flash is non saturating. The relative extent of the signal induced by the second flash as a function of $t_d$ indicates the time course of the recovery for recharging. This is the case when the charging induced by the second flash at $t_d$ is proportional to the number of places which were discharged in $t_d$.

## 3. Results and discussion

The relative value $\phi = \Delta\varphi_{II}(t_d)/\Delta\varphi_{II}(t_d \rightarrow \infty)$ measured optically, $\Delta\varphi_{II}^{opt}$, and electrically, $\Delta\varphi_{II}^{el}$, in dependence of the dark time $t_d$ between a double flash are depicted in fig. 2. The value of $\phi$ is biphasic. The smaller slow phase ($\sim$ 20%) is due to the reoxidation of plastohydroquinone by Chl-$a_I$ [3]. The dominant fast phase (80%) can be described by a first-order kinetic. The time for $\phi/2$ of the fast phase is 0.6 msec or $k = 1150$ sec$^{-1}$ for both types of measurements:

$$\tau^{opt}(\Delta\varphi_{II}\text{recov}) = \tau^{el}(\Delta\varphi_{II}\text{recov}) = 0.6 \text{ msec}$$

Both values are in very good agreement with the electron transfer time from $H_2O$ to the PQ pool (see above). The results indicate:

1. The agreement between the transfer time from $H_2O$ to PQ via $X$–320 and the optically and electrically measured recovery time for $\Delta\varphi_{II}$ is in accordance with our earlier assumption of a *vectorial* electron transfer from $H_2O$ to $X$–320. This transfer could be discussed, how-

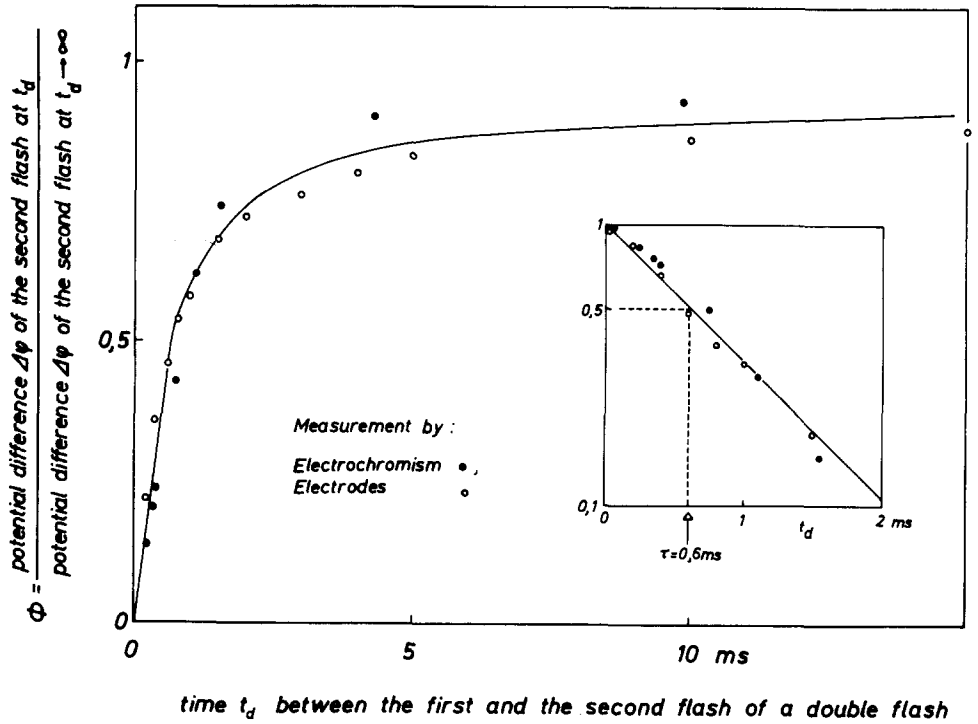


Fig. 2. Electrical potential difference of the second flash in dependence on the darktime $t_d$ between the first and second flash of a double flash. The potential difference at $t_d$ is related to the maximal value at $t_d \rightarrow \infty$ (●–●–●) Potential difference measured by electrochromism; (○–○–○) potential difference measured by electrodes. *Inlay*: Plot of the *fast* phase in a log scale. *Subject*: Chloroplast of spinach. Chlorophyll content $2 \times 10^{-5}$ M in 2 ml solution, $10^{-2}$ M tricine, pH 8, $10^{-2}$ M KCl, $10^{-2}$ M sucrose, $10^{-4}$ M benzylviologen as electron acceptor, 20°C. Flash duration $2.10^{-5}$ sec (single turnover flash). Far red background light 720 nm, $5 \times 10^4$ erg/cm$^2$ sec.

ever, to take place in the *plane* of the membrane. But this possibility has been shown to be very unlikely [4–6]. Furthermore it was recently shown that the electrical measurement with electrodes responds only to potentials with a component perpendicular to the membrane [19, 20]. Therefore the electrically measured recovery time in fig. 2 supports also that the vectorial electron transfer from $H_2O$ to $X$–320 occurs *across* the membrane and not in the plane;

2. The agreement between the results of the optical and electrical measurement in fig. 2 supports in an additional way that the field indicating absorption changes are indicating electrical potentials;

3. The proportionality between the extent of the optical and electrical signal in fig. 2 confirms our earlier conclusion that the potential is *linearly* indicated by the optical changes [8, 15].

## Acknowledgements

We thank Dr. G. Renger for constructive criticism of the manuscript. We thank Miss Ch. Fleckenstein for technical assistance. This work was supported by tie Deutsche Forschumgsgemeinschaft.

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