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Oxidation–reduction potentials of photosynthetic intermediates

Although the concept of two photoreactions in photosynthesis operating in series is supported by various types of evidence, some data about the oxidation–reduction potential of intermediates and, consequently, about the free energy changes in the reaction chain between the two primary photoacts are difficult to reconcile with the concept as it is usually formulated. The standard oxidation–reduction potential, E'_0, (at pH 7) of P700, the primary reductant of System 1, has been found to be +430 – +450 mV in isolated spinach chloroplasts and in chloroplast preparations[1,2]. However, conflicting data have been reported about the E'_0 of Q, the primary oxidant of System 2, which was variously reported to be −35 mV, about +200 mV or even only 40–60 mV lower than that of P700 (refs. 3–5). Since better knowledge of the energetics of the photosynthetic reactions is essential for an understanding of the mechanism of photosynthesis, we have measured spectrophotometrically the redox levels of some of the constituents of the photosynthetic chain in intact algae during illumination.

Fig. 1 shows the relation between the redox levels of cytochrome *f* and P700, measured by means of absorbance difference spectrophotometry, in the red algae *Porphyridium cruentum* and *Porphyra umbilicalis*. The oxidation levels of both compounds increased with intensity in a similar way as shown for cytochrome *f* in Fig. 2. The data corresponded to an equilibrium constant, $K = 9.5$, independent of the intensity, or a difference in oxidation–reduction potential, $\Delta E'_0 = 58$ mV. Similar but less precise results were obtained with the green alga *Chlorella vulgaris* and the blue-green alga *Anacystis nidulans* ($\Delta E'_0$, approx. 75 and approx. 60 mV, respectively). The above numbers are in close agreement with results recently reported by MARSHO AND KOK[6] for illuminated spinach chloroplasts but would indicate a higher E'_0 than reported by KATOH[7] for cytochrome *f* isolated from *Porphyra tenera* and by BENDALL[8] for cytochrome *f* in pea chloroplasts. Measurement of $\Delta E'_0$ in the dark which for isolated chloroplasts was reported to be higher[6] was not possible, because P700 and cytochrome *f* were in the reduced state in darkness.

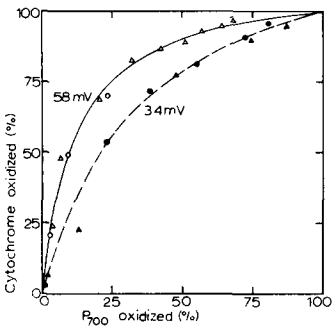
3-(3,4-Dichlorophenyl)-1,1-dimethylurea (DCMU) lowered the apparent $\Delta E'_0$ to 30–35 mV for *P. cruentum* and *Pa. umbilicalis* (Fig. 1). The cause of this phenomenon is not clear. One explanation might be that the apparent K (and $\Delta E'_0$) is decreased because the establishment of an equilibrium between the components of different reaction centers is in some way prevented. For the same reason, the $\Delta E'_0$ measured without DCMU may have been too low already. If DCMU only acts at System 2, then its effect on the equilibrium would imply separate reaction chains up to Q (*cf.* ref. 9).

We have used a similar method to obtain information on the $\Delta E'_0$ between cytochrome *f* and Q in *P. cruentum*. The redox level of Q was calculated from the fluorescence yield of chlorophyll *a* at various intensities of illumination. Fluorescence was excited by weak modulated green light of constant intensity; accessory "actinic" illumination was provided by a continuous beam, in a way similar to that described by DUYSENS AND SWEERS[10]. Preillumination with blue light was employed in order

Abbreviation: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

to obtain maximum conversion of Q′ into Q (*cf.* refs. 10, 11) and to maintain aerobiosis.

The fluorescence yield corresponding to complete reduction of Q was measured in the presence of DCMU. The yield in moderately intense blue (System 1) actinic light was assumed to correspond to complete oxidation of Q. This yield was approximately independent of the intensity between about $0.1 \cdot 10^{-9}$ and $0.5 \cdot 10^{-9}$ Einstein$\cdot$sec$^{-1}\cdot$cm^{-2}; at lower and higher intensities the fluorescence yield was higher. The corresponding redox levels of cytochrome *f* were measured under identical experimental conditions by means of absorption difference spectrophotometry with the same apparatus as used for measuring fluorescence yields (*cf.* ref. 12). Green (System 2) light, at any intensity, was unable to effect an observable reduction of cytochrome *f*.



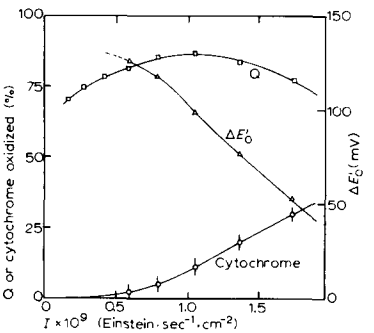


Fig. 1. Relation between the redox levels of P700 and of cytochrome in *P. cruentum* without (○—○) and with $5 \cdot 10^{-5}$ M DCMU (●--●) and in *Pa. umbilicalis* without (△—△) and with $5 \cdot 10^{-5}$ M DCMU (▲--▲). The data were obtained by measuring the steady-state difference in absorbance (light *minus* dark) at 705 nm and 420 nm, respectively, upon illumination with red light (640 nm, half band width 25 nm). The intensity was varied between $0.3 \cdot 10^{-9}$ and $3 \cdot 10^{-9}$ Einstein$\cdot$sec$^{-1}\cdot$cm^{-2}. The redox levels were calculated by comparison with the maximum absorbance changes obtainable in the presence of DCMU with saturating red or blue light. The solid and dashed lines give the calculated curves for $\Delta E'_0 = 58$ and $\Delta E'_0 = 34$ mV, respectively.

Fig. 2. "Steady-state" oxidation–reduction levels of cytochrome *f* (○—○) and Q (□—□) in *P. cruentum* and the corresponding apparent $\Delta E'_0$ (△—△), as a function of intensity of actinic light. The vertical bars indicate the error of measurement in the redox level of cytochrome, measured spectrophotometrically. The redox level of Q was calculated from the fluorescence yield of chlorophyll *a* as described in the text, assuming a probability of energy transfer between reaction centers (*P*) of 0.55 (ref. 17). The wavelength of actinic illumination was 640 nm. A 2-min preillumination with blue light (about 400–480 nm) of approx. $1.3 \cdot 10^{-9}$ Einstein$\cdot$sec$^{-1}\cdot$cm^{-2} was applied. Fluorescence excitation light was 560 nm, intensity about $4 \cdot 10^{-11}$ Einstein$\cdot$sec$^{-1}\cdot$cm^{-2}.

This indicates that, within the error of measurement ($\pm$ 3 %), in darkness the cytochrome was completely reduced.

The results of a typical experiment are given in Fig. 2. The wavelength of actinic light in this experiment was 640 nm, which gave approximately equal excitation of Systems 1 and 2. Intensities below about $0.6 \cdot 10^{-9}$ Einstein$\cdot$sec$^{-1}\cdot$cm^{-2} did not give a measurable oxidation of cytochrome; at higher intensities the cytochrome became partially oxidized, up to 30 % at $1.7 \cdot 10^{-9}$ Einstein$\cdot$sec$^{-1}\cdot$cm^{-2}. Q was 70 % oxidized at very low intensity; its oxidation level increased with intensity to 80–85 % at $1.0 \cdot 10^{-9}$ Einstein$\cdot$sec$^{-1}\cdot$cm^{-2} and slowly decreased again at higher intensities. These data give an equilibrium constant $K = 120$–140, corresponding to $\Delta E'_0 = 125$ mV

at $0.6 \cdot 10^{-9}$ Einstein$\cdot$sec$^{-1}\cdot$cm^{-2}. The "true" equilibrium constant (to be measured at lower intensities) may of course have been higher. The lower apparent $\Delta E'_0$ at intensities above $0.7 \cdot 10^{-9}$ and the gradual accumulation of reduced Q above $1 \cdot 10^{-9}$ Einstein$\cdot$sec$^{-1}\cdot$cm^{-2} (and also in strong blue light) probably reflect a shift away from the equilibrium levels. Oxygen evolution, measured polarographically, was found to be linear with intensity up to about $1.5 \cdot 10^{-9}$ Einstein$\cdot$sec$^{-1}\cdot$cm^{-2}, and the rate-limiting step is thought to be between cytochrome *f* and Q (ref. 13).

Similar experiments with *Pa. umbilicalis* and *C. vulgaris* yielded $\Delta E'_0 \geqslant 100$ and $\geqslant$ approx. 90 mV, respectively, at low intensities of illumination. With *C. vulgaris* a far-red preillumination was applied; the redox level in far-red light was assumed to correspond to complete oxidation of Q, for the same reason as discussed for blue light for *P. cruentum*.

The above results indicate that in the intact photosynthesizing cell a $\Delta E'_0$ of at least 180 mV is generated between light reactions 1 and 2. This corresponds to $K = 1100$ for P700 and Q, considerably more than reported by JOLIOT *et al.*[5] for isolated spinach chloroplasts. As follows from the above discussion, this number may be considered a lower limit. A complication in the interpretation of the results is the possibility of a coupled phosphorylation step in the chain between P700 and Q. As has been discussed[5], this could cause an intensity dependence of K; the intensity dependence actually observed by us may have been partly due to this effect. The assumption of such a phosphorylation step with a P/2*e* ratio of 1 (for a discussion of this point see refs. 14, 15) then would increase the $\Delta E'_0$ of the uncoupled reaction by about 160 mV, *i.e.* to 340 mV or more. An E'_0 of P700 of +440 mV then gives a maximum E'_0 for Q of +100 mV, in agreement with the results of CRAMER AND BUTLER[3] rather than with those of KOK *et al.*[4]. Experiments to measure the $\Delta E'_0$ between cytochrome and Q in the presence of carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone were not successful because of complicated effects of this uncoupler on the fluorescence behavior which are at present not well understood (*cf.* ref. 16).

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