ON THE OXIDATION-REDUCTION POTENTIAL OF THE PHOTOPRODUCED REDUCTANT OF ISOLATED CHLOROPLASTS

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Several reports have appeared in recent years on the determination of the redox potential of the photoproduced reductant of isolated chloroplasts. Wessels (1954), after a detailed study of a large number of Hill oxidants, concluded that only compounds with a redox potential above zero could be reduced even under strictly anaerobic conditions. The discovery of the photoreduction of TPN (San Pietro and Lang, 1958) led to the conclusion that the photoproduced reductant of isolated chloroplasts must possess a redox potential lower than -0.32 volts. The demonstration of the photoreduction of ferredoxin (Tagawa and Arnon, 1962) lowered the estimated value of the redox potential of the reductant even further to around -0.43 volts. Kandler (1960) had suggested earlier that the redox potential of the photoproduced reductant of isolated chloroplasts had to be about -0.6 volts.

The availability of low-potential dipyridyl compounds (E_0' as low as -0.55 volts), which could be photoreduced, has made it possible to obtain a better estimate of the actual redox potential of the reductant of isolated chloroplasts. In order to determine conveniently the concentrations of the reduced and oxidized forms of the dipyridyl compounds, the molar extinction coefficients of these compounds had to be determined. The molar extinction coefficient of reduced paraquat[4] (synonomous with methyl viologen; 1, 1'-dimethyl, 4, 4'-dipyridylium diiodide) was found to be 4.26×10^4 at 396 mμ and

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2.84 x 10^4 at 385 mμ. In the studies reported here the higher extinction coefficient at 396 mμ was used. The experimental procedure for the determination of extinction coefficients was as described under Table I, except for the omission of the chloroplasts and for the addition of 0.05 ml. of 1% freshly dissolved sodium dithionite in 0.3 M TRIS-HCl buffer, pH 7.8, in the side arm of the Thunberg tube. After complete evacuation of the tube, dithionite was added, and the spectrum recorded on a Model 14 Cary spectrophotometer. In the case of triquat (1,1'-trimethylene, 2,2'-dipyridylium dibromide), a stable spectrum of the reduced form of the compound was obtained at a pH of 12.7, giving an extinction coefficient of 1.4 x 10^4 at 387 mμ. Due to the high negative redox potential of triquat ($E_0' = -0.55$ volts) (Homer, et al., 1960), the reduction by dithionite could be expected to

TABLE 1

PHOTOREDUCTION OF LOW-POTENTIAL DIPYRIDYL COMPOUNDS BY ISOLATED CHLOROPLASTS

Additions	Maximal Reduction	E_h (pH 7.8)
	Per cent	volts
Paraquat (39 μg chlorophyll)	84	-0.49
Triquat (39 μg chlorophyll)	20	-0.51
Triquat (78 μg chlorophyll)	30	-0.53
Triquat (55 μg chlorophyll) no catalase, no ethanol	0	-

The reaction mixture contained in total volume of 3.0 ml in μmoles: Tris-HCl (pH 7.8), 45; paraquat, 0.6; triquat, 3.0; ethanol, 700; catalase (Boehringer - crystalline), 50 μg; once-washed chloroplasts from Swiss chard, containing the indicated amount of chlorophyll, prepared as previously described (Avron, 1961) but omitting ascorbate from the washing medium. The reaction mixture was placed in a modified Thunberg tube fitted with an optical glass cuvet (1-cm), evacuated to 25 mm Hg with continuous tapping for 2 minutes, and closed. Spectra were recorded on a Cary Model 14 spectrophotometer before and after illumination with 80,000 lux of white light at 20°C.

proceed with difficulty yielding an unstable free radical. Under strictly anaerobic conditions at a pH of 12.7, however, the free radical of reduced triquat was stable for more than 20 minutes. Since the reduction of dipyridyl compounds does not involve hydrogen (Michaelis and Hill, 1933; Homer, et al., 1960), the molar extinction coefficient would be expected to be independent of hydrogen ion concentration and to be valid at pH 7.8 of the chloroplast reactions.

Zweig, et al. (1965) have shown that diquat (1,1'-ethylene, 2,2'-dipyridylium dibromide), with a redox potential of - 0.35 volts (Homer, et al., 1960) could be almost quantitatively reduced by illuminated isolated chloroplasts. For this reason, diquat is not the most suitable compound for the determination of the redox-potential of the photoproduced reductant, since a slight increase in reduction above 90% introduced a large experimental error from the Nernst equation. Two other dipyridyl compounds with more negative redox potentials than diquat were chosen, paraquat, E_0' = -0.47 volts and triquat, E_0' = -0.55 volts. As is seen in Table I, paraquat is almost quantitatively photoreduced by isolated chloroplasts under strictly anaerobic conditions, whereas triquat with the more negative redox-potential to a lesser extent. Maximum photoreduction was achieved within 20 minutes' illumination. Oxidation of triquat in the dark occurred at a much slower rate than photoreduction, whereas photoreduced diquat and paraquat remained stable for at least 30 minutes in the dark. The calculated redox potential of the endogenous photoproduced reductant ranged from -0.49 v to -0.53 v. The experimental redox potential (E_h (pH 7.8)) is not identical with E_0', since the redox state of the endogenous reductant is not known. If one were to assume that one-half of the reductant is oxidized when the measurements are made, the E_0' of the reductant will be approximately -0.5 v. Although ferredoxin is reportedly the most reducing constituent isolated from spinach chloroplasts (E_0', pH 7.55 = -0.432 v) (Tagawa and Arnon, 1962), one must assume that a reductant with a more negative redox potential must be present in Swiss chard chloroplasts.

The effect of chlorophyll concentration on the photoreduction of paraquat is significant (Table I), supporting the hypothesis that an endogenous reductant exists. The absolute requirement for the absence of oxygen is also illustrated in Table I. Davenport (1963) and Zweig, et al. (1965) have also reported that the photoreduction of diquat proceeds best in the presence of a catalase-ethanol peroxide trap. This is also suggestive of the production of hydrogen peroxide during the auto-oxidation of dipyridyl compounds (Davenport, 1963).

The observations that the experimentally obtained value for the redox potential of the photoproduced reductant in isolated chloroplasts is approximately -0.5 volts and that ferredoxin was not required for the photoreduction of diquat (Zweig, et al., 1965), have led to the conclusion that ferredoxin may not be the endogenous reductant with the most negative oxidation-reduction potential.

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