A REQUIREMENT FOR REDUCED PLASTOQUINONE A IN THE HILL REACTION OF EXTRACTED CHLOROPLASTS

P. M. Wood and F. L. Crane
Department of Biological Sciences
Purdue University
Lafayette, Indiana

Received June 14, 1965

Studies of the requirement for plastoquinone A for the restoration of Hill reaction activity after extraction of chloroplasts with isooctane consistently revealed that the crude extract restored more activity than saturating levels of plastoquinone A (PQA). Similar stimulatory effects of crude extracts have previously been noted by Bishop (1959) and Ogren and Krogmann (1963). Investigation of this effect revealed that the active material in the extract was the reduced form of plastoquinone A ($PQAH_2$).

Whole chloroplasts were prepared from spinach by grinding 200 g. of deveined leaves in 400 ml. of 0.35 M NaCl in a Waring blendor for 30 sec. The homogenate was filtered through cheesecloth and centrifuged 5 minutes at 700 xg. The supernatant was recentrifuged at 1600 x g for 10 min, and the sediment was immediately frozen in a dry ice-acetone bath and lyophilized not longer than 4 hours. The lyophilized chloroplasts were extracted with 20 ml. spectral grade isooctane per 10 mg of chloroplast chlorophyll. Extraction was carried out at room temperature ($22^{o}c$) for 10 min. in a glass homogenizer loosely fitted with a teflon pestle (cf. Krogmann and Olivero, 1962).

Supported in part under grant GM 10741 from the National Institute of General Medical Research and a David Ross Grant from Purdue Research Foundation. F. L. Crane is Research Career Awardee from U. S. Public Health Service.

Homogenization was by hand. The chloroplasts were harvested on a sintered glass filter and washed with isooctane. Solvent remaining in the chloroplasts was removed by a current of N_2.

Quinones and extracts were added to the dry extracted chloroplasts in 1.0 ml isooctane, followed by evaporation of the solvent.

Hill reaction activities were assayed by determination of oxygen release, in the light in the presence of potassium ferricyanide or TPN; the oxygraph (Gilson Medical Electronics) apparatus uses a rapidly oscillating platinum electrode polarized at 0.64 volts relative to a saturated calomel electrode, with amplifier and recorder for measuring the current resulting from the reduction of oxygen which diffuses to the electrode. In some cases the photoreduction of TPN or ferricyanide was assayed spectrophotometrically on an aliquot of the same reaction mixture used for the oxygen release determination. All assays were carried out in the oxygraph reaction cell, which was enclosed in a jacket through which water at 22° was rapidly circulated. The light source was a 100 W tungsten lamp focused through a substage condensor.

Thin layer chromatograms of the extract using 30% heptane in chloroform as the developing solvent revealed plastoquinone A and small amounts of plastoquinones C and D and tocopheryl quinone by methylene blue spray, and several pink spots by ferric chloride-dipyridyl spray (Dilley, 1964). The major ferric chloride positive spots correspond to reduced plastoquinone A and α-tocopherol. Material isolated from the chromatogram in the region corresponding to reduced plastoquinone A was the only fraction which could replace the crude extract in restoration of activity. The tocopherols, and plastoquinones C, D, or E are inactive. If the reduced plastoquinone A obtained from the chromatogram is oxidized to the quinone by standing it is no longer active as extract factor, but still shows activity equivalent to plastoquinone A.

The oxidized factor also shows chromatographic and spectral properties

Table I

Reactivation of the Hill Reaction after Isooctane Extraction

Treatment	PQA Removed (μmole)	PQA Added (μmole)	$PQAH_2$ Removed (μmole)	$PQAH_2$ Added (μmole)	O_2 released (μmole) mg.chl. TPN as acceptor	O_2 released (μmole) mg. chl. FeCN as acceptor
Unextracted	-	-	-	-	0.80	1.13
Extracted	0.11	None	0.20	None	0.06	0.21
+ PQA	0.13	0.20	0.23	None	0.25	0.34
+ $PQAH_2$	0.11	None	0.20	0.20	0.46	0.49
+ PQA + $PQAH_2$	0.12	0.15	0.22	0.20	0.41	0.44
+ Extract*	0.11	0.12**	0.19	0.21**	0.40	0.45

* Extract was added at a level equal to that in the original chloroplasts.

** These values are the amounts of the quinone or hydroquinone in the extract which was added (i.e. are not supplementary to the extract). Basal reaction mixtures: (1) 25μmole Tris-HCl buffer pH 7.2, 1.0 mg TPN, PPNR (saturating), chloroplasts with approximately 0.150 mg chlorophyll, H_2O to 1.5 ml. (2) 25 μmole Tris-HCl buffer pH 7.2, 1.0 μmole potassium ferricyanide, chloroplasts with about 0.150 mg. chlorophyll, H_2O to 1.5 ml. The reaction was carried out for 3.0 min. at 22°C.

equivalent to plastoquinone A.

A comparison of the effects of plastoquinone A, the extract and plastoquinone A reduced with borohydride is shown in Table 1. The extract and reduced plastoquinone A restore more activity than a saturating level of plastoquinone A. The amounts of reduced and oxidized plastoquinone A in the extract determined after chromatography and elution of these substances and reoxidation of the reduced plastoquinone A are comparable to the amounts of these substances added. The residue contained 0.007 μmoles PQA per mg chlorophyll and no reduced PQA was detected. In other experiments TPN photo reduction as well as oxygen release was found to follow a similar pattern.

Pretreatment of chloroplasts with high levels of ferricyanide in the dark at pH 8.0 has been shown to inhibit the Hill reaction activity of these chloroplasts (Brewer and Jagendorf, 1965). We find that this ferricyanide treatment causes oxidation of all the endogenous reduced plastoquinone A as determined by isooctane extraction. This oxidation may partly explain the inhibitory effect of ferricyanide preincubation as shown in Table II. Further studies will be needed to determine whether the reduced

Table II

Affect of Ferricyanide Preincubation on Photoreduction of Ferricyanide

	Plastoquinone A Content		O_2 Released μmoles/mg.chl./3 min. Ferricyanide as Acceptor
	% Oxidized	% Reduced	
Tris chloride treatment	62	38	1.97
Ferricyanide treatment	94	6	1.01

Treatment: Control in 0.005 M Tris Cl pH 8.0 for 15 min. at 4°C. experimental in 0.05 M potassium ferricyanide and 0.005 M Tris chloride at pH 8.0. Reaction mixture as in Table I with 10 μmoles ammonium chloride. Oxidized and reduced PQA determined after separation by thin layer chromatography.

plastoquinone A requirement is an artifact resulting from the physical character of the reduced plastoquinone A which permits penetration to an active site, or whether it represents the need for a redox poising system to initiate photoreduction. This latter possibility is consistent with the finding that at least 50% of the original activity remains after oxidation of the endogenous reduced plastoquinone A ferricyanide. As an alternative it is possible that two pathways of electron flow are involved.

References

Bishop, N. I., (1959) Proc. Nat. Acad. Sci. (Wash) 45, 1696.

Brewer, J. M. and Jagendorf, A. T., (1965) Plant Physiol., 40, 303.

Dilley, R. A. (1964) Anal. Biochem. 7, 240.

Krogmann, D. W. and Olivero, E., (1962) J. Biol. Chem., 231, 277.

Ogren, W. L. and Krogmann, D. W. (1963) in Photosynthetic Mechanisms of Green Plants, National Academy of Sciences - National Research Council, p. 174.