PLASTOQUINONE REDUCTION IN ILLUMINATED CHLOROPLASTS

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Plastoquinone (PQ) is a lipid soluble benzoquinone which is localized in chloroplasts (Crane 1959a). Its structure has been defined by Trenner et al. (1959) as 2,3-dimethyl-5-solanosylbenzoquinone. The quinone is present in spinach chloroplasts at a level of 0.1 mole per mole of chlorophyll. In our preparations of chloroplasts (Crane 1959a) most of the plastoquinone is present in the oxidized form, although in some preparations it is partially in a reduced form. Any reduced quinone present can be converted to the oxidized form by treatment of the chloroplast suspension with 0.03 M potassium ferricyanide in the dark.

When chloroplasts containing oxidized plastoquinone are exposed to bright light (1000 footcandles, Tungsten) at 25°C. the endogenous plastoquinone is reduced up to 80% within five minutes (cf. Table 1). A slight reduction may occur in the dark. On further incubation in light or dark the reduced plastoquinone is slowly converted to the oxidized form.

When resuspended boiled chloroplasts are exposed to light the plastoquinone is not reduced.

It has been necessary to develop a selective extraction technique in order to observe the changes in plastoquinone. For this assay chloroplasts are incubated in 3.5 ml. of solution.

Table 1

Reduction of Endogenous Plastoquinone in Illuminated Chloroplasts

Incubation		Observed Change at 254 mμ.	Correction*	Plastoquinone mg. PQ per mg. chlorophyll	
Time	Condition			Oxidized	Total
0	dark	0.104	.027	.077	.081
5 min.	light	0.047	.027	.020	.080
5 min.	dark	0.123	.067	.056	.089

Spinach chloroplasts (0.5 mg. chlorophyll) incubated in 0.001 M potassium chloride and 0.25 M sucrose at 25°C. in a total vol. of 3.5 ml. Reaction stopped by adding 1.0 ml. 15% trichloroacetic acid. Plastoquinone extracted with isooctane and the oxidized form determined by borohydride reduction in ethanol. Total PQ determined after oxidation by passage through decalso.

After incubation under the desired conditions 1 ml. of 15% trichloroacetic acid is added to stop the reaction. Four ml. of isooctane are added and the mixture is shaken for 30 min. on a reciprocal shaker. An aliquot of the isooctane phase is then dissolved in ethanol for determination of oxidized plastoquinone by the borohydride reduction assay (Crane 1959b) in which the quinone is measured by following the change from oxidized to reduced at 254 mμ. Total (oxidized + reduced) plastoquinone in this extract is determined by passing the extracted lipids through a decalso column to oxidize any reduced quinone present and to remove certain impurities which interfere in the assay. Elution from a decalso column (4 g. decalso per mg. of chlorophyll in the sample) is made successively with: (1) 25 ml. iso-

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* Correction for interfering compound with change in absorbancy from 230 to 290 mμ. during borohydride treatment.

octane, (2) 25 ml. 10% ethyl ether in isooctane and (3) 25 ml. 100% ethanol in isooctane. Plastoquinone is found in eluate 2. The total plastoquinone found by this procedure is the same as is found in an ethanol ether extract of total chloroplast lipids.

Another compound which shows a decrease in absorbancy at 254 mμ. after treatment with borohydride is also present in isooctane extracts. It can be distinguished from PQ in that it shows decrease in absorbancy from 290 to 230 mμ. whereas PQ shows an increase in the 290 mμ. region with an isobestic point at 276 mμ. Therefore, any decrease at 276 mμ. upon borohydride treatment can be used as a measure of this other compound. The interferring compound is separated from PQ on the decalso column and is eluted in fraction 3. We have used the change in absorbancy at 254 mμ. produced by borohydride treatment of fraction 3 to correct for the interference of this compound in the original extract. The amount of the compound showing decrease in absorbancy from 290 to 230 mμ. declines in chloroplasts ex-

Table 2

Reduction of Lipids in Chloroplasts Exposed to Light

Incubation		Reduced lipids μeg. ferric chloride reduced per mg. chlorophyll*
Time	Condition	
0	-	3.00
5'	light	3.30
5'	dark	3.01

Incubation conditions as described in Table 1.

* Calculated for a two electron reaction as in the oxidation of plastoquinone.

posed to light and increases in the dark. Lipid materials other than plastoquinone are also reduced in chloroplasts in light. When the isooctane extract is titrated with the ferric chloride-dipyridyl reagent of Emmerie and Engel (1938) the amount of ferric chloride reducing materials is found to increase in light much more than if only plastoquinone were reduced (cf. Table 2).

The evidence that plastoquinone undergoes oxidation reduction changes together with Bishop's (1959) demonstration that plastoquinone is required to restore the Hill reaction in solvent treated chloroplasts indicates a function for this quinone in electron transport associated with photosynthesis.

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